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CHROMOSOME LENGTH IN RELATION TO TRANSMISSION FREQUENCY OF MAIZE TRISOMES[1]

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Received December 3, 1942

TRISOMIC inheritance was first reported in maize by McCLINTOCK (1929) in a study of segregating progenies derived from a spontaneous triploid (RANDOLPH and McCLINTOCK 1926). Later McCLINTOCK and HILL (1931) identified the primary trisome of chromosome 10, the shortest member of the maize chromosome complement which carries the *r-g* linkage group, and certain general observations on the known primary trisomic types of maize were made by RHOADES and McCLINTOCK (1935). The present study was undertaken to compare the breeding behavior of the eight available primary trisomic types in maize.

MATERIAL AND METHODS

The trisomic stocks used in this study were procured from the MAIZE GENETICS COOPERATION, DEPARTMENT OF PLANT BREEDING, CORNELL UNIVERSITY, Ithaca, New York. The stocks were grown by DR. L. F. RANDOLPH in 1940 to isolate trisomic plants and establish cultures that did not possess B-type chromosomes. The trisomic plants so isolated were crossed by various stocks carrying known genetic markers and also by certain commercial inbred lines. The progenies of these crosses were grown in the summer of 1941. In the text and tables, these F_1 progenies are designated by a number which refers to the particular chromosome present in triplicate in the female parent, and the male parent, which was either recessive for a gene carried by a member of the trisome or an inbred line, is also specified.

Acetocarmine smear preparations were used to study the details of meiosis in the microsporocytes of these F_1 plants. The sporocyte samples were fixed in a mixture of one part of acetic acid to two parts of absolute alcohol. Somatic chromosome counts were made from root tips fixed in CRAF (RANDOLPH 1935), sectioned in paraffin and stained with crystal violet.

TRANSMISSION FREQUENCIES OF THE VARIOUS TRISOMES

If all sporocytes in a trisomic plant possessed a trivalent, and if two of the three members of the trivalent passed to one pole at the first meiotic anaphase

[1] These studies were made in the DEPARTMENTS OF BOTANY AND PLANT BREEDING, COLLEGE OF AGRICULTURE, CORNELL UNIVERSITY, Ithaca, New York. The writer wishes to express his sincere appreciation to DR. L. F. RANDOLPH of the DIVISION OF CEREAL CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE and the NEW YORK STATE COLLEGE OF AGRICULTURE, Ithaca, New York, for advice and assistance throughout the course of this problem and for the many helpful suggestions in the interpretation and presentation of the material.

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with the third member passing to the opposite pole, 50 percent of the gametes would contain the n number of chromosomes and 50 percent would contain n+1 chromosomes. However, in the cross of 2n+1 × 2n, considerably less than 50 percent of the F_1 individuals were trisomic. The reduction from 50 percent is greater when the trisomic individual is the male parent. This has been established for maize, Datura, Matthiola, tomato and other plants. Since transmission of n+1 gametes through the male parent is limited by differential pollen tube growth, analysis of other factors also involved can best be made in connection with their transmission through the female gametophyte.

It has been found that the degree of transmission by the seed parent varies with the particular chromosome which is present in triplicate. In Datura (BLAKESLEE and AVERY 1938) the number of trisomic plants in the progeny of different trisomic parents varies from 3 percent in stocks of the 19·20 trisome to 32.7 percent transmission for the 23·24 chromosome. These transmission differences could not be correlated with differences in chromosome size. It has been shown that microspores carrying n and n+1 chromosomes apparently are formed in equal numbers in Datura (BLAKESLEE and AVERY 1938), and it was suggested that the same proportions also are present among the megaspores of these plants. It was also suggested that the deficiency of 2n+1 plants in the progeny was due to elimination of the extra chromosome or of cells containing the extra chromosome during some stage or stages of development of the gametophyte or zygote.

LESLEY (1928, 1932) found ovule transmission of the extra chromosome in tomato trisomics to vary from 18 percent to 32 percent. The loss of the extra chromosome at meiosis and zygotic lethality, suggested by the presence of many small inviable seeds in fruits of trisomic types, were thought to account for deficiency of ovule transmission of the extra chromosome.

In studies on *Nicotiana sylvestris*, GOODSPEED and AVERY (1939) found a variation in the transmission of the extra chromosome through the ovules of primary trisomic plants which ranged from 16.3 percent to 28.8 percent. This variation was not considered to be significant. The failure to approach the theoretical value of 50 percent transmission was attributed to the lagging of the extra chromosome in the meiotic divisions.

The deficiency of trisomics in the progeny of trisomic plants of Matthiola was attributed by FROST (1927) to disturbances of the reduction division and subsequent development of gametes and embryos caused by the presence of the extra chromosome, which created sufficient unbalance in the delicately adjusted developmental processes to produce lethal effects.

MCCLINTOCK and HILL (1931) reported a transmission frequency of the extra chromosome of 33.06 percent in a cross of 2n+1 by 2n involving trisome 10 in maize. The deviation from the expected 50 percent was explained on the basis of irregularities at meiosis. Genetic evidence was also presented to show that 35 percent of the eggs carried n+1 chromosomes. RHOADES (1933) found a transmission frequency of 31 percent in a study involving trisome 5 in maize.

In the present study, random samples of 26 to 177 kernels were taken from

the ears derived from the trisomic stocks of the Maize Genetics Cooperation. In many of these stocks (trisomic for chromosomes 2, 3, 7, 8, or 10) samples from two or more ears were used. These ears in each case came from sister plants pollinated by a different male parent, either a known genetic stock or a commercial inbred line. The seeds were carefully germinated in trays. Chromosome numbers were determined from root tips taken from each seedling before it was transplanted to the field. Because the pollen parent was diploid and selection of seed was random, these determinations indicated the percentage transmission of the extra chromosome through the egg of the 2n+1 female parent.

The frequency of occurrence of 2n+1 individuals in the F_1 progeny of the 2n+1 × 2n crosses was not the same for all trisomics investigated. Also, the frequency of occurrence of 2n+1 individuals differed among the progeny of female parents possessing the same chromosome in triplicate (see trisomics 2, 3, and 10, table 1). There are several possible explanations for this variation. Inadequate sampling of certain stocks was a contributing factor. Likewise, certain genotypes among the genetically different stocks may have tended to promote a higher or lower rate of transmission. Variability of environmental conditions also might have influenced the results.

The various maize trisomes differed appreciably in the frequency with which they were transmitted to their progeny through the female gametophyte. The lowest transmission frequency was found to be 22 percent in a stock of trisome 9 and the highest transmission frequency was 52 percent in stocks of

TABLE 1

The viability of various trisomic stocks and the percentage of trisomics found in the progenies of 2n+1×2n *crosses.*

TRISOMIC STOCK	EAR FERTILITY %	NUMBER OF KERNELS	NUMBER OF KERNELS GERMINATED	NUMBER OF TRISOMICS	% TRISOMICS
2×LF inbred	95–100	148	134	65	49
2×inbred II	95–100	53	44	16	36
2×C11 inbred	95–100	100	93	48	52
2×*lg1*	90– 95	58	52	20	39
3×LF inbred	50+ poor ear	48	34	12	35
3×inbred II	75– 90	59	52	17	33
3×*lg2*	95–100	96	91	41	45
5×inbred II	95–100	101	89	46	52
6×*su2*	95–100	159	155	59	38
7×LF inbred	Tiny ear	26	22	10	45
7×inbred II	80– 90	81	58	23	40
8×LF inbred	90– 95	150	146	47	32
8×*j*	95–100	51	45	12	27
9×*wx*	100	123	113	25	22
10×LF inbred	95–100	177	149	38	26
10×*v18*	95–100	52	49	18	37

trisomes and 2 and 5. This unequal transmission of different trisomes through the egg is in agreement with data reported by other workers in Datura, Matthiola, Lycopersicum, Nicotiana, and other plants. The following explanations have been advanced by various workers to account for these differences:

Viability is decreased as a result of the unbalance caused by the extra chromosome. This unbalance may result in the abortion of some of the n+1 eggs or 2n+1 zygotes at different stages in ontogeny as suggested in Datura, Lycopersicum, and Matthiola.

The replacement of certain of the megaspores containing n+1 chromosomes in the chalazal position by megaspores containing the balanced number has been suggested by McCLINTOCK and HILL (1931).

The observed frequencies may be actual gametic frequencies, the extra chromosome being lost in the meiotic divisions.

With reference to viability, McCLINTOCK and HILL (1931) emphasized that the failure to find transmission frequencies approaching the theoretical 50 percent apparently was not due to lack of viability of the n+1 female gametes or 2n+1 plants, since trisomic plants were obtained with almost perfectly filled ears. In the mature ears of maize the regularity of the rows and kernel positions permits undeveloped kernels to be readily detected. In the present experiments an estimate was made of the fertility of all the ears of trisomic plants from which seed was taken. In table 1 these are noted as percent fertility. A rating of 95–100 percent indicates that the fertility is normal or indistinguishable from that of an average diploid ear. Since most of the ears used showed normal fertility, it may be concluded that any differential viability prior to seed germination plays an insignificant role in determining the proportion of trisomic individuals which appear in progenies.

The failure of 2n+1 seeds to germinate probably had no significant influence on the relative frequency of trisomics. Fourteen hundred and eighty-two seeds were started. Of these 1326 or 90 percent germinated. It is probably true that a greater percentage of the smaller seeds failed to germinate. Because the lighter seeds are more often trisomic than the heavier ones in all stocks used in the experiments (EINSET unpublished), the percentage of trisomics present in progenies may be slightly low. Of the 1326 seedlings that were transplanted to the field, 38 or 2.8 percent died before reaching maturity. Twenty of these were disomic, seven were trisomic, two were monosomic, one plant had 19 chromosomes plus a fragment chromosome, and in six the chromosome constitution was not determined. Most of these plants died soon after the transplanting, probably because of injuries sustained in handling. There was no indication of lessened viability caused by the presence of an extra chromosome.

The possibility that the basal megaspore, if it contains the extra chromosome, does not function to produce the embryo sac but is replaced by one containing an n complement in a certain percentage of the cases was suggested by McCLINTOCK and HILL (1931). No indication of such substitution of megaspores was found by SINGLETON and MANGELSDORF (1940) in a study of gametic lethals on the fourth chromosome of maize. RHOADES (1942) followed the development of 200 embryo sacs in a study concerned with an abnormal type of

chromosome 10 and found that no replacement of chalazal megaspores occurred. To test the possibility of megaspore replacement in trisomic maize, a study of embryo sac development in a large number of ovules would be necessary. This study was not undertaken. However, chromosome number determinations of microspores are readily obtainable, and if the behavior of the extra chromosome during meiosis is comparable in mega- and microsporogenesis, a comparison of observed ratios of n: n+1 microspores in the parent trisomic plants with 2n and 2n+1 individuals in the progeny of these would indicate whether megaspore replacement was occurring. This comparison is shown in table 2. Although the chromosome counts of microspores were obtained from 2n+1 individuals in the F_1 progeny, the agreement in the ratio of n:n+1 microspores with 2n:2n+1 individuals is very close. If such a comparison is valid, one may conclude that the ratios of 2n:2n+1 individuals represent the true gametic ratios. It would also suggest that genic variability, known to be present among the different megaspores, female gametophytes and 2n+1 individuals, does not materially influence viabilities. The possibility that the observed ratio of n:n+1 microspores in a given sample may vary with the stage at which counts were made should be considered (McCLINTOCK 1938). If the rate of development of n and n+1 spores through prophase and metaphase is not comparable, then a sample in which only a small percentage of the microspore nuclei have entered division might give a ratio differing from that

TABLE 2

Progeny and microspore counts of trisomic plants and occurrence of univalents at metaphase I.

TRISOMIC STOCK	% TRISOMICS IN PROGENIES OF TRISOMIC PLANTS	N*	% MICROSPORES WITH n+1 CHROMOSOMES	N*	% MICROSPOROCYTES WITH UNIVALENTS AT METAPHASE I	N*
2×LF inbred	49	132	50	212	30	247
2×inbred II	37	43	47	149	17	202
2×C11 inbred	52	93	—	—	—	—
2×*lg1*	39	52	44	93	12	153
3×LF inbred	35	34	—	—	32	190
3×inbred II	33	52	—	—	—	—
3×*lg2*	45	91	41	167	24	152
5×inbred II	52	89	50	198	14	209
6×*sw2*	38	155	34	109	28	109
7×LF inbred	45	22	50	193	30	136
7×inbred II	40	58	—	—	24	110
8×LF inbred	32	146	37	113	40	267
8×*j1*	27	45	36	132	—	—
9×*wx*	22	113	23	218	44	214
10×LF inbred	26	149	34	190	49	372
10×*v18*	37	49	33	109	37	300

* N equals the total number of observations.

obtained from a sample in which most of the spores have completed this division. Since the figures presented in table 2 represent composites from many anthers, in which different percentages of spores had completed the division, the possibility of such an effect presumably was eliminated.

The evidence presented above indicates that the observed differences in the frequencies of transmission of the extra chromosome through the egg, which characterized the various trisomic stocks in maize, were due to the difference in frequency with which n and n+1 megaspores were formed.

CHROMOSOME LENGTH AND TRANSMISSION FREQUENCY

The frequency of trisomics in progenies of trisomic plants and the percentage of microspores with n+1 chromosomes, presumably a measure of the frequencies of n and n+1 gametes, in stocks of different trisomics are summarized in table 3. The relative length at pachytene of each chromosome involved in this study, as determined by LONGLEY (1939), is indicated in the table. These eight chromosomes are arranged in three groups according to their relative lengths—namely, long (chromosomes 2, 3, and 5), medium (chromosomes 6, 7, and 8), and short (chromosomes 9 and 10).

It is evident that there is a positive correlation between the length of a

TABLE 3

Chromosome lengths and frequencies of trisomics in F_1 progenies, microspores with n+1 chromosomes, and microsporocytes with univalents at metaphase I.

RELATIVE CHROMOSOME LENGTHS*	TRISOMIC STOCK	% TRISOMICS IN PROGENIES OF TRISOMIC PLANTS	N†	% MICROSPORES WITH n+1	N†	% MICROSPOROCYTES WITH UNIVALENTS AT METAPHASE I	N†
80	2	47	320	48	454	20	602
74	3	45	91	41	167	28	342
73	5	52	89	50	198	14	209
Averages for long chrs.		48		46		21	
60	6	38	155	34	109	28	109
56	7	41	80	50	193	26	246
57	8	31	191	36	245	40	267
Averages for medium chrs.		37		40		31	
52	9	22	113	23	218	44	214
45	10	28	198	34	299	43	672
Averages for short chrs.		25		29		44	

* Relative lengths at pachytene, with chromosome 1 equal to 100, based on measurements on 33 Indian varieties of corn from the United States (LONGLEY, 1939).

† N equals the total number of observations.

chromosome and the frequency with which n+1 gametes involving different chromosomes are transmitted through the female gametophyte. Plants trisomic for the three longer chromosomes (chromosomes 2, 3, and 5) transmitted the extra chromosome through nearly 50 percent of its eggs; plants trisomic for the chromosomes of medium length (chromosomes 6, 7, and 8) transmitted the extra chromosome through 37 percent of the eggs, and plants trisomic for the short chromosomes (chromosomes 9 and 10) averaged only 25 percent transmission of the extra chromosome through the eggs. In some cases variation from the mean within each group was rather pronounced. Nevertheless a positive correlation is obvious.

MEIOTIC BEHAVIOR OF THE TRISOMES

The behavior of the various trisomes was studied during microsporogenesis to determine whether the observed chromosome length-transmission frequency correlation could be related to some specific behavior of the chromosomes during meiosis. An explanation of the variability found in different stocks was likewise desired. Results obtained in such studies should be applicable to megasporogenesis, since the progeny and microspore counts showed such close agreement.

According to McCLINTOCK and HILL (1931) approximately two-thirds of the microsporocytes in plants trisomic for chromosome 10 contained nine bivalents and one trivalent at metaphase I; in the other sporocytes there were ten bivalents and a univalent. The univalent, when present, behaved very irregularly and often was not included in either telophase nucleus. Trivalents, on the other hand, seemed to disjoin regularly, two from one, in the first division of meiosis, and no irregularity was observed in division II. Consequently, it would be expected that 2n+1 plants having large numbers of microsporocytes with ten bivalents plus one univalent would produce fewer n+1 spores, and thus gametes, than plants having a lower number of such microsporocytes. The data presented in table 3 indicate that this was true.

The observed differences in univalent frequency at metaphase I were traced to differences in the frequency of disassociation of one member of the trivalent configurations during late prophase I. This was accomplished by examining progressive prophase stages between pachytene and metaphase I, the material being obtained from adjacent florets of the same tassel, fixed at the same time. The following observations were recorded on material from a plant trisomic for chromosome 3(3×LF inbred):

STAGE OF MEIOSIS	% OF CELLS SHOWING A UNIVALENT	TOTAL NUMBER OF CELLS OBSERVED
Pachytene	2- 4	112
Diplotene	7-11	104
Middle diakinesis	31	45
Late diakinesis	30	100
Metaphase I	32	190

The maximum values for cells showing a univalent at pachytene or diplotene were obtained by assuming that all doubtful cells, which were relatively few in number, contained the univalent. The pachytene observations indicated that in 96–98 percent of the microsporocytes synapsis was normal. Trivalents were formed regularly through the two-by-two synapsis of the three homologues at different levels. As the prophase progressed through diakinesis to metaphase I, a higher frequency of univalents was found.

From these data it may be concluded that most of the univalents observed at metaphase I result from the separation of one member from the trivalent complex either at diplotene or early diakinesis. This desynapsis could be attributed to the failure of chiasma formation following DARLINGTON'S (1933) theory of post-diplotene association by chiasmata.

At metaphase I, the three homologues were present either as a trivalent or as a bivalent and a univalent. Only rarely were three univalents observed. When a univalent is present at this stage, it may be found almost anywhere in the cell. Observations were made on 859 sporocytes possessing ten bivalents plus a univalent. In 132 or 15.4 percent of the cells the univalent chromosome was on the equatorial plate along with the bivalent chromosomes. In 136 cells (15.8 percent) the univalent was present in the cytoplasm outside the spindle In the remaining 591 cells (68.8 percent) the univalent was definitely off the equatorial plate but within the limits of the spindle.

The two sister chromatids of a univalent may show signs of separation as early as metaphase I or very early anaphase I. This is especially true if the univalent is located on the equatorial plate or near the plate within the spindle.

When a trivalent is present, disjunction at anaphase I is usually two from one. When a univalent is present, it may be included in one of the anaphase groups of disjoining bivalents and subsequently be included in a telophase nucleus, or it may fail to be included in either telophase nucleus. Data on the percentage of microsporocytes with univalents at metaphase I (table 3) and microspores with n+1 chromosomes indicate that in trisomic stocks involving the longer chromosomes most of the univalents were being transmitted to the microspores. This was substantiated by the low percentage of lagging at anaphase I (table 4). The medium and short chromosomes, when present as univalents, were lost to the telophase nuclei and not included in the microspores with a much higher relative frequency (table 3). The relative amount of lagging of univalents at anaphase I in stocks of trisomes 9 and 10 was also greater than in the stock of trisome 2 (table 4). When a univalent lags, in many instances the two-sister chromatids making up the univalent separate or show signs of separation. Rarely, however, do the separated chromatids (monads) reach the poles. Among 149 cells at late anaphase I none was found with ten dyads plus one monad at each pole. In 96 of these cells there were ten dyads at one pole and 11 dyads at the opposite pole. In 25 cells a univalent, either undivided or in the process of division, was present at the equatorial plate region. In the remaining 28 cases, the univalent occupied a position closer to one pole than the disjoining ten dyads, or was located outside the spindle

altogether. It seems probable, therefore, that although the two halves of a univalent often show signs of separation at metaphase I and anaphase I, this separation is ordinarily completed too late for the monads to be included in one or both of the telophase nuclei. However, RHOADES (1940) found that occasionally at least the daughter univalents of an unpaired telocentric chromosome did reach the poles in time to be included in the interphase nuclei. This was made evident by the observation of daughter univalents in prophase II. In the trisomic material a number of chromosome counts were made at pro-

TABLE 4

Frequency of univalents and micronuclei at meiosis I and II in various trisomic stocks, expressed in percentage.

UNIVALENT AND MICRONUCLEI FREQUENCY, IN %	TRISOMIC STOCK									
	2×LF	N*	3×LF	N	9×*wx*	N	10×LF	N	10×*v*18	N
Total number of univalents present										
Diakinesis	28	275	30	145	32	195	—		33	100
Metaphase I	30	247	32	190	44	214	49	372	37	300
Detached univalents or micronuclei										
Anaphase I	3	60	—		41	39	29	45	19	32
Telophase	4	53	31	71	15	126	24	151	13	80
Interphase	—		—		—		19	136	—	
Metaphase II	—		11	93	—		—		38	101
Anaphase II	—		15	13	—		—		12	33
Telophase II	—		13	85	—		—		15	300
Spore quartets with micronuclei	—		—		—		6	100	7	57
Spores with n+1 chromosomes	50	212	—		23	218	34	190	33	109

* N equals number of sporocytes, number of pairs of cells in division II or number of quartets of spores.

phase II, but in no case were ten dyads plus a monad found in each of the daughter cells. Univalents that were included in the telophase I nuclei passed undivided to one of the two poles of the spindle.

At telophase I the univalent or its two separated halves that were not included in the daughter nuclei may be found anywhere within the cell, either at the plate region, near one of the telophase nuclei or elsewhere in the cytoplasm. This excluded chromatin may be present as a compact chromatin mass or becomes organized as a distinct micronucleus. Often there are two such compact

chromatin masses or micronuclei, probably representing the two sister halves of a former univalent. Occasionally a fragment or a chromatin bridge was noted.

In general, the second meiotic division was freer of irregularities than the first. Relatively little chromosome lagging was observed. Observations were made on paired or daughter cells at telophase II. Occasionally one of these two cells contained a compact chromatin mass or a micronucleus. In other cases, each of the two cells contained one such body, presumably representing the sister halves of a former univalent. Certain paired cells were observed in which there were two micronuclei in one daughter cell and none in the other.

BEHAVIOR OF MICRONUCLEI IN TRISOMIC PLANTS

During the meiotic divisions univalent chromosomes which remain in the cytoplasm may be transformed into micronuclei, or they may degenerate and subsequently disappear. It is also possible that such excluded chromosomes or micronuclei are not permanently "lost" but may be reincorporated in the macronuclei. If this happens, the frequency of apparent loss of the univalent during division I in trisomic plants cannot be utilized to estimate the frequency of occurrence of n+1 chromosome complements in spores and gametes. WATKINS (1924) reported on univalent behavior at microsporogenesis in F_3 plants from a cross of *Triticum turgidum* × *T. vulgare*. The univalents arrived at the equatorial plate after the bivalents and split, but the halves were often not included in the daughter nuclei. Many of these lagging chromatids were included in the nuclei after the second meiotic division. Micronuclei decreased in frequency as the microspore developed, and the assumption was made that odd chromosomes degenerated during pollen development. CHANDLER, PORTERFIELD, and STOUT (1937) in a study of triploid types of *Lilium tigrinum* found that "the lagging chromatin elements in the cytoplasm undergo noticeable and rapid disintegration." "Microcysts" or compact chromatin masses were observed to form directly from lagging chromosomes or by degeneration of micronuclei. Later, these chromatin masses disappeared. They also noted that fragments or lagging elements near the equatorial plate region were swept to the periphery of the cell as the phragmoplast expanded at the telophase I. These lagging elements remained at the periphery of the cell and either degenerated or formed micronuclei.

MYERS (1941a) studied the behavior of univalents in certain plants of *Lolium perenne* showing abnormal meiotic behavior. Lagging univalents divided equationally in all observed cases, and it was concluded that a majority of the daughter half-chromosomes from anaphase I laggards were included in the daughter nuclei prior to the interphase stage. Similar behavior was observed in a hybrid of *Phleum pratense* × *Phleum subulatum* (MYERS 1941b). However, in the latter case the univalents were more regularly oriented on the equatorial plane along with the bivalents than in the plants of *Lolium perenne*, and there was also a more regular equational division of the univalents at anaphase I.

Table 4 summarizes the data on the occurrence of univalents and micronuclei at different stages of meiosis in several stocks of maize. There was a decrease in the percentage of cells showing lagging chromosomes or micronuclei from telophase I to the spore quartet stage. The question arises as to whether this decrease was due to incorporation of micronuclei into macronuclei, to disintegration of micronuclei, or to the incorporation of the micronuclei in separate microcytes. The answer may be found in table 4. The trisomic culture 10 × LF inbred may be used as an example. Chromosome counts at the first microspore division showed that 34 percent of these spores were n+1. If the extra chromosome was lost in 30 percent of the microsporocytes at division I, 35 percent of the microspores would be expected to have an extra chromosome. It is, of course, realized that there may be some division of the univalent in the first followed by chromatid lagging in the second division, but this and other anomalous types of behavior are relatively rare as previously shown. Anaphase I showed 29 percent of the cells with a lagging univalent or a univalent in the cytoplasm. Thus the percentage of anaphases with detached univalents corresponded very closely to the expected 30 percent loss of univalents calculated from the known fact that 35 percent of the spores had n+1 chromosomes.

From anaphase I to the spore quartet stage a steady decrease in number of micronuclei was observed. In culture 10 × LF, only 6 percent of the quartets possessed micronuclei. In other cultures this same tendency was observed. Microspores with micronuclei were considerably less frequent than was to be expected from the observed irregular meiotic behavior of the univalents. The incorporation of the micronuclei into macronuclei will not explain this reduction. If this occurred, the number of microspores with n+1 chromosomes would be considerably greater than was observed. Since there was no evidence of microcyte formation or other related phenomena that might account for their disappearance, it was concluded that the micronuclei disintegrated in the cytoplasm during the interim from anaphase I to spore formation.

CHROMOSOME VARIANTS OTHER THAN PRIMARY TRISOMICS

Several chromosome types other than primary trisomics appeared in the crosses of 2n+1 × 2n plants. Among the 1916 plants in which chromosome counts were made for the primary purpose of identifying trisomic individuals, there were, in addition to 658 trisomics, five monosomics (19 chromosome plants), one plant with 19 chromosomes plus a fragment chromosome, one with 20 plus a fragment, three with 21 plus a fragment, two haploids and three triploids. The chromosome fragments present in five of these plants were not supernumerary B chromosomes but presumably represented deletions or additions involving members of the normal complement. The frequency of 19 chromosome plants or monosomics, six among 1916, was higher in the trisomic stocks than was expected, since they previously have been observed infrequently in maize. Unfortunately, five of these six plants were extremely weak and died soon after transplanting to the field, and it was not possible to identify the

individual monosomics. In order to determine whether or not the observed frequency of monosomics in these trisomic cultures differed significantly from their spontaneous occurrence in diploid maize, it would be necessary to make chromosome determinations or critical genetical tests of large numbers of plants produced from very carefully germinated seeds of normal diploid ears. In ordinary field plantings, most monosomics probably would be eliminated early in ontogeny due to a lack of vigor; and this probably accounts in part at least for the very low frequency of monosomics ordinarily observed. Of the five plants with fragments which were found, only one, a plant with 21 chromosomes plus a fragment, grew past the seedling stage. It was extremely weak and produced no tassel or ear. Fragment types have also been derived from progenies of trisomics in Zea, Datura, Lycopersicum, and *Nicotiana sylvestris*, but apparently have not resulted in the unexpected lethality which in these experiments accompanied the presence of chromosome fragments. The frequency of haploidy in these stocks, two among 1916 plants, does not differ significantly from the normal spontaneous frequency of approximately one in 2000 (RANDOLPH unpublished). The four triploid plants which occurred in the trisomic progenies cannot definitely be said to have originated spontaneously, since the trisomic stocks were grown in proximity to tetraploid maize with which they might have been contaminated.

DISCUSSION

Among the various plant species in which trisomic inheritance has been studied, specificity of effect of different trisomes may be apparent not only in the phenotypic appearance of the individual, but also in its breeding behavior. The extra chromosome, theoretically expected to be present in 50 percent of the gametes, has been found in most of the forms studied to be transmitted rarely through the pollen and transmitted with varying frequencies through the egg. The specific effects of different chromosomes in Datura are manifested in differential elimination of the extra chromosomes and differential reduction of viability of spores, gametes, zygotes, embryos, and seeds containing the extra chromosome. Zygotic lethality and reduced seed viability are interpreted as being due to influences of the trisomes similar to those which are partially responsible for the differences in the transmission of different chromosomes through the egg, that have been noted in Matthiola, Lycopersicum, and *Nicotiana sylvestris*.

In Zea no such explanation can be advanced. Any unbalance causing lethality of spores, eggs, or zygotes would be detected in the lowered fertility of the ear. The ears of trisomic plants, however, are wholly fertile. Therefore, the observed differences in transmission frequency of specific maize trisomes must mean that spores with n and n+1 chromosomes are not formed in equal numbers as in Datura microspores, but in whatever ratio the progenies indicate. That this is true of the megaspores as well as the microspores is indicated by the strong positive correlation between microspore counts and transmission frequencies of the various trisomes through the seed parent. This same expla-

nation has been suggested as a partial cause of the differences observed in Matthiola, Lycopersicum, and *Nicotiana sylvestris*. Meiotic irregularities due to the presence of a univalent resulted in the frequent loss of the extra chromosome. That the presence of a trivalent, on the other hand, imposes greater regularity is supported by various investigations.

The data presented in this paper indicate that in maize the extra chromosome is present in the male and female gametes from 22 percent to 52 percent of the time, depending on the particular trisome that is involved. There is a definite positive correlation between the length of the chromosome and the frequency with which it is present in spores or transmitted through the egg. Differences in the frequency of loss of the extra chromosome at meiosis is a function of the length of the extra chromosome (varying from relative values of 80 to 45 in the trisomic stocks of maize studied). BELLING (1924) studied the chromosome numbers of 52 microspores of triploid hyacinths which contain long, medium, and short chromosomes. He concluded that the chromosomes of different length were distributed according to chance. His data, however, suggested that there was some slight tendency for the shorter chromosomes to be lost more often, or to be less frequently present in the microspores than were the medium and long chromosomes.

Data which are here presented on the frequency of trivalents at metaphase I indicate that there are more trivalents present when the trisome is made up of the longer chromosomes of the set. Similar observations were made by BELLING (1925) in certain triploid hyacinths, by NEWTON and DARLINGTON (1929), and DARLINGTON and MATHER (1932) in triploid Tulipa, and by others. In *Nicotiana sylvestris*, GOODSPEED and AVERY (1939) found no differences in trivalent frequencies among the four different trisomics which they studied. Neither did they find length differences in the extra chromosomes, nor significant differences in transmission frequencies of these four chromosomes when present in triplicate.

It has been shown that these differences in trivalent frequencies at diakinesis and metaphase I in the maize material here studied are due to desynapsis of one of the three homologous members of the trivalent. On the basis of DARLINGTON'S theory of post-diplotene association by chiasmata, this early separation of previously synapsed homologues in the late prophase would be due to the failure of one of the three homologous chromosomes to form a chiasma with either of the other two. The frequency with which chiasmata, that would prevent desynapsis, might be formed would then be conditioned by the variation in length of the pairing chromosomes and in the number of chiasmata formed in any given length (DARLINGTON and MATHER 1932).

DARLINGTON, MATHER, and others have published extensively on chiasma behavior and find, in general, a correlation between chromosome length and the number of chiasmata formed. In diploid maize as well as in trisomic maize (DARLINGTON 1933; MATHER 1939) the Stenobothrus type (DARLINGTON and DARK 1932) of relationship exists, the correlation being roughly linear, but the shortest chromosomes have a proportionately higher chiasma frequency

than the longer ones. DARLINGTON (1933) found relatively little reduction in chiasma frequency through terminalization in maize. BEADLE (1933), however, found an average of 3.7 chiasmata per bivalent at diplotene but only 1.8 at diakinesis in maize.

In the trisomic stocks of maize involving the long chromosomes the opportunity for any two chromosomes to be synapsed over relatively long regions is greater than it is among the shorter chromosomes, and for this reason it follows that the opportunity for chiasma formation is greater; there is less desynapsis and consequently more trivalents at metaphase I. If there is little terminalization in maize, or the same relative amount for long and short chromosomes, it is improbable that this phenomenon would account for the greater prevalence of univalents in plants trisomic for the shorter chromosomes.

Briefly, when a short chromosome is present in triplicate, fewer trivalents and more univalents are present at metaphase I. Irregular assortment of the univalents in anaphase I results in their frequent elimination, and this elimination of the univalents reduces the number of spores and gametes containing the extra chromosome. Since n and n+1 gametes and the zygotes which they form are equally functional, there are fewer trisomic individuals in the progeny of plants trisomic for the shorter chromosomes than in the progeny of those trisomic for the longer chromosomes.

There was a relatively great amount of variability in transmission frequency among various stocks of the same trisome as well as a discrepancy between the value for trisome 5 (31 percent) reported by RHOADES (1933) and the value here reported (52 percent). This variability cannot be correlated with chromosome length, but may possibly be explained on the basis of variability in the number of univalents at metaphase. RHOADES (1933) found a low value (10–15 percent) for the frequency of univalents at metaphase, although transmission was only 31 percent. Different samples from the same trisomic stocks sometimes showed rather extreme fluctuations in univalent frequency. One sample of 8×LF had 30 percent of the sporocytes with univalents at metaphase I, another had 48 percent of the sporocytes with univalents. In the case of 2×LF inbred the counts indicated that the frequency of univalents at metaphase I was 30 percent. Chromosome counts in microspores and progenies of this trisomic indicated that 50 percent of the gametes were of the n+1 type. If a large percentage of the univalents is lost, how is it possible to find such high frequencies? LESLEY (1928), working with 3n tomatoes, observed considerable variation in meiotic chromosome behavior in samples examined the same day, even within the same anther. The literature dealing with abnormalities in meiosis caused by heat, cold, and other environmental effects is extensive. STOW (1927) found very marked decrease in conjugation at a temperature of 25–30°. MATSUURA (1937) noted more univalents in metaphase I under high temperature conditions. OEHLKERS (1935) also made extensive studies of the varying effects of temperature on crossing over and chiasma formation. It seems, therefore, that the observed variability within

stocks may have been caused by environmental effects, or possibly by genic effects which might have altered either the usual synaptic relations of the trisome or more likely chiasma formation and the occurrence of univalents at meiosis. More extensive studies in a carefully controlled environment should throw additional light on this question.

SUMMARY

A study of the breeding behavior of eight of the ten primary trisomes of maize (trisomes 1 and 4 were not studied) revealed marked differences in the frequency with which the extra chromosome was transmitted to the progeny through the egg. Transmission frequencies determined by somatic chromosome counts of 1916 plants ranged from approximately 50 percent for the longer chromosomes to approximately 25 percent for the shorter chromosomes, and the chromosomes of intermediate lengths exhibited intermediate transmission frequencies.

Failure of the extra chromosome to be transmitted to 50 percent of the progeny in $2n+1 \times 2n$ crosses apparently was not due to replacement of $n+1$ megaspores by spores containing the n number, since there was very close agreement in specific crosses between the percentage of microspores containing the extra chromosome and the percentage of trisomic plants in the progeny. Neither was it due to differential viability of zygotes, since the ears of trisomic plants were as well filled as those of their disomic sibs, and their seeds were equally viable.

The failure of the extra chromosome to be transmitted to 50 percent of the progeny through the egg apparently was due to the elimination of the extra chromosome as a univalent in the meiotic divisions.

Desynapsis of one member of the trivalent to form a univalent occurred more frequently in trisomic plants involving the shorter chromosomes than in those involving the longer chromosomes. This desynapsis took place in late prophase; there was regular two-by-two synapsis of the three homologues at pachytene.

It is suggested that this desynapsis was due to the failure of chiasma formation between the chromosome that desynapsed and either of the other two, chiasmata presumably preventing complete separation of synapsed homologues during late prophase. On the basis of the chromosome length-chiasma frequency correlation, this failure would be more frequent in trivalents composed of short chromosomes than in trivalents made up of long chromosomes.

Univalents frequently lagged in the meiotic divisions and failed to be incorporated in the daughter nuclei. Thereafter they sometimes formed micronuclei, but more often they disappeared, probably as a result of disintegration in the cytoplasm.

In a population of 1916 plants derived from the cross $2n+1 \times 2n$, 658 plants (34.34 percent) were trisomic, two were haploids, six were monosomics, four were triploids, and five carried fragment chromosomes in addition to the complement of normal chromosomes.

LITERATURE CITED

BEADLE, G. W., 1933 Further studies of asynaptic maize. Cytologia **4**: 269–287.

BELLING, J., 1924 The distribution of chromosomes in the pollen-grains of a triploid hyacinth. Amer. Nat. **58**: 440–446.

1925 Homologous and similar chromosomes in diploid and triploid hyacinths. Genetics **10**: 59–71.

BLAKESLEE, A. F., and A. G. AVERY, 1938 Fifteen-year breeding records of 2n+1 types in *Datura Stramonium*. Cooperation in Research, Carnegie Inst. Washington Publ. **501**, 315–351.

CHANDLER, C., W. M. PORTERFIELD, and A. B. STOUT, 1937 Microsporogenesis in diploid and triploid types of *Lilium tigrinum* with special reference to abortions. Cytologia, Fujii Jubilee Volume: 756–784.

DARLINGTON, C. D., 1933 The origin and behavior of chiasmata. VII. *Zea Mays*. Z.I.A.V. **67**: 96–114.

DARLINGTON, C. D., and S. O. S. DARK, 1932 The origin and behavior of chiasmata. II. *Stenobothrus parallelus*. Cytologia **3**: 169–185.

DARLINGTON, C. D., and K. MATHER, 1932 The origin and behavior of chiasmata. III. Triploid Tulipa. Cytologia **4**: 1–15.

FROST, H. B., 1927 Chromosome-mutant types in Stocks (*Matthiola incana* R. Br.). I. Characters due to extra chromosomes. J. Hered. **18**: 474–486.

GOODSPEED, T. H., and P. AVERY, 1939 Trisomic and other types in *Nicotiana sylvestris*. J. Genet. **38**: 381–458.

LESLEY, J. W., 1928 A cytological and genetical study of progenies of triploid tomatoes. Genetics **13**: 1–43.

1932 Trisomic types of the tomato and their relation to the genes. Genetics **17**: 545–559.

LONGLEY, A. E., 1939 Knob positions on corn chromosomes. J. Agric. Res. **59**: 475–490.

MCCLINTOCK, B., 1929 A cytological and genetical study of triploid maize. Genetics **14**: 180–222.

1938 The fusion of broken ends of sister half-chromatids following chromatid breakage at meiotic anaphases. Missouri Agric. Expt. Sta. Research Bull. **290**: 1–48.

MCCLINTOCK, B., and H. E. HILL, 1931 The cytological identification of the chromosome associated with the r-g linkage group in *Zea Mays*. Genetics **16**: 175–190.

MATHER, K., 1939 Chiasma frequencies in trisomic maize. (Abstract) Genetics **24**: 104.

MATSUURA, H., 1937 Chromosome studies on *Trillium kamtschaticum Pall*. V. Abnormal meiotic divisions due to high temperatures. Cytologia, Fujii Jubilee Volume: 20–34.

MYERS, W. M., 1941a Variations in chromosomal behavior during meiosis among plants of *Lolium perenne*. Cytologia **11**: 388–406.

1941b Meiotic behavior of *Phleum pratense*, *Phleum subulatum*, and their F_1 hybrid. J. Agric. Res. **63**: 649–659.

NEWTON, W. C. F., and C. D. DARLINGTON, 1929 Meiosis in polyploids. I. J. Genet. **21**: 1–16.

OEHLKERS, F., 1935 Untersuchungen zur Physiologie der Meiosis. I. Z. Bot. **29**: 1–53.

RANDOLPH, L. F., 1935 A new fixing fluid and a revised schedule for the paraffin method in plant cytology. Stain Tech. **10**: 95–96.

RANDOLPH, L. F., and B. MCCLINTOCK, 1926 Polyploidy in *Zea Mays* L. Amer. Nat. **60**: 99–102.

RHOADES, M. M., 1933 An experimental and theoretical study of chromatid crossing over. Genetics **18**: 535–555.

1940 Studies of a telocentric chromosome in maize with reference to the stability of its centromere. Genetics **25**: 483–520.

1942 Preferential segregation in maize. Genetics **27**: 395–407.

RHOADES, M. M., and B. MCCLINTOCK, 1935 The cytogenetics of maize. Bot. Rev. **1**: 292–325.

SINGLETON, W. R., and P. C. MANGELSDORF, 1940 Gametic lethals on the fourth chromosome of maize. Genetics **25**: 366–390.

STOW, I., 1927 A cytological study on pollen sterility in *Solanum tuberosum* L. Japan. J. Bot. **3**: 217–238.

WATKINS, A. E., 1924 Genetic and cytological studies in wheat. I. J. Genet. **14**: 129–171.

MULTIPLE ALLELES IN COMPLEMENTARY SEX DETERMINATION OF HABROBRACON[1]

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INTRODUCTION

SEX determination in most animals and in many dioecious plants is of the backcross type. Females are recessive, *mm* (in cytological terms XX); males heterozygous dominant, *Mm* (YX or OX); or males may be recessive, *ff* (ZZ); females heterozygous dominant, *Ff* (WZ or OZ). The dominant factor segregating as a unit in the digametic sex, *M* (Y or O) in the male, *F* (W or O) in the female, is usually an absence of sex-producing genes, its effectiveness depending upon autosomal sex-producers.

This scheme does not hold for bees, wasps, and ants or for many other invertebrates in which males are normally haploid, produced parthenogenetically, females diploid and usually produced from fertilized eggs. If a haploid set of chromosomes be doubled, the ratio of sex determining genes will not be changed and hence the insect developing from the fertilized egg should be of the same sex as that developing from the unfertilized.

This remained a puzzle until 1933 when it was shown (WHITING 1933) that sex determination in the parasitic wasp *Habrobracon juglandis* (Ashmead) is complementary, that there are different kinds of haploid males similar in appearance but containing different sex factors (xa+A and xb+A), and that the diploid complex of the female represents the heterozygous "double dominant" combination of two different male complexes (xa+xb+2A). This complementary theory was proved when the sex differentiating alleles were shown to be linked with the mutant gene fused, *fu*, affecting antennae, legs, and wings. Diploid males, which have been known in Habrobracon for several years, are homozygous for the sex alleles (2xa+2A and 2xb+2A).

This principle may be illustrated by the following example. An xa/xb female homozygous for the autosomal recessive gene veinless, *vl*, but heterozygous for fused and having fused associated with xb may be crossed with a non-veinless xb fused male (table 1, A). The recessive veinless serves to mark the haploid sons which are fused and non-fused in equal numbers. The diploid offspring, whether males of females, will be non-veinless. Among the daughters,

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TABLE 1

Ratios of offspring expected according to various arrangements of sex alleles in crosses of veinless, vl, females heterozygous for fused, fu, by fused males.

	MOTHER	FATHER	HAPLOID SONS		DAUGHTERS xa/xb or xa/xc and xb/xc		DIPLOID SONS AND EXCESS BAD EGGS xa/xa or xb/xb	
			vl	*vl fu*	+/*fu*	*fu*/*fu*	+/*fu*	*fu*/*fu*
A	$\frac{vl\ \text{xb}\ fu}{vl\ \text{xa}\ +}$	$\frac{\text{xb}\ fu}{}$	5	5	9	1	1	9
B	$\frac{vl\ \text{xa}\ fu}{vl\ \text{xb}\ +}$	$\frac{\text{xa}\ fu}{}$	5	5	9	1	1	9
C	$\frac{vl\ \text{xa}\ +}{vl\ \text{xb}\ fu}$	$\frac{\text{xa}\ fu}{}$	5	5	1	9	9	1
D	$\frac{vl\ \text{xb}\ +}{vl\ \text{xa}\ fu}$	$\frac{\text{xb}\ fu}{}$	5	5	1	9	9	1
E	$\frac{vl\ \text{xb}\ fu}{vl\ \text{xa}\ +}$	$\frac{\text{xc}\ fu}{}$	5	5	10	10	0	0

xa/xb, the non-crossovers will be wild type, the crossovers fused, but among the diploid sons, xb/xb, the reverse condition obtains. Since crossing over between x and fused is about 10 percent, fused will show great deficiency among the daughters, great excess among the diploid sons. If both parents have fused associated with xa, the offspring occur in the same ratio (table 1, B), but if fused is associated with xa in one parent and with xb in the other, the ratios of fused among the two types of diploid offspring are reversed (table 1, C and D) giving deficiency of fused among the daughters, excess among the sons. Thus there are four different arrangements possible for x and fused but only two phenotypically separable ratios.

Actually the diploid sons are of very low viability, many failing to hatch from the egg, and fused individuals are somewhat less viable than non-fused. Moreover, other things being equal, the proportion of haploid sons among total progeny depends upon the number of eggs remaining unfertilized. This is roughly about one-third under standard laboratory conditions so that the haploid males tend to be about equal to their sisters. An unfertilized egg may be said to be more fertile, more likely to hatch into a viable insect, than a fertilized egg.

The statements made above have been found to apply to matings between closely related individuals from inbred laboratory stocks. In contrast, when females are outcrossed to males of unrelated stocks, no diploid sons are produced, and daughters are twice as numerous as from closecrosses. The diploid sons and excess unhatchable fertilized eggs are replaced by females. To explain these conditions hypotheses such as differential maturation (WHITING

1935) and multiple factors (SNELL 1935) were suggested. Both of these theories were later shown to be untenable, failing to fit certain data, especially those collected by BOSTIAN.

BOSTIAN (1939), crossing heterozygous females to fused males, showed that whenever diploid males occurred, fused appeared sex-linked, but if diploid males were lacking, fused and non-fused females were produced in approximately equal numbers. According to the differential maturation theory, outcrossing should not affect sex-linkage of fused. BOSTIAN closely inbred his stock for more than twenty generations, in the last 11 by sibling matings. Females heterozygous for fused were always crossed to fused males, and lines were continued from fraternities of the "outcross" type—in other words, from those with no diploid males and with non-fused and fused females in equal numbers. In spite of inbreeding, he was able to maintain "outcrosses" as the majority type of his fraternities, but in every generation a few "closecrosses" appeared with diploid males and with obvious sex-linkage of fused. BOSTIAN's failure to establish a line free from sex-linkage of fused disproved the multiple factor theory according to which sex differentiation should have been shifted from xa/xb to a second pair of factors, za/zb. The true explanation for BOSTIAN's "outcross" fraternities lay in the fact that he was maintaining a third allele (xc) in the x series by selection, and the few "closecross" fraternities which he obtained represented crossovers between x and fused. WHITING (1939) had tentatively suggested the multiple allele hypothesis and later (1940a) reviewed all published data showing them to be consistent with it. Subsequently (WHITING 1941) quadruple alleles were proved in crosses involving two different stocks.

Fraternities of the "closecross" type may now be called two-allele fraternities, those of the "outcross" type three-allele fraternities. A third allele brought into the cross by a fused male, xc *fu* for example, always masks sex-linkage, since all diploid offspring are heterozygous for x and hence female, fused and non-fused in approximately equal numbers (table 1, E). Diploid males reappear with inbreeding, and their presence is always associated with obvious sex-linkage of fused as shown by BOSTIAN (1939).

Different inbred stocks may have the same sex alleles, xa/xb and xa/xb, in which case all matings result in two-allele fraternities; they may differ by a single factor, xa/xb and xa/xc, in which case half of the matings result in two-allele fraternities, half in three-allele; or they may have no sex factor in common, xa/xb and xc/xd, in which case all matings result in three-allele fraternities.

According to the multiple allele theory any heterozygote for two members of the series, xa, xb, xc, etc., is female, any homozygote or azygote (haploid) is male. Given n alleles in the series, there should be possible n different haploid males, n corresponding diploid males and $(n^2-n)/2$ females.

PROCEDURE

After it has been shown that one or both of the sex differentiating factors of

two stocks are different, the allelism of the series of sex factors may be established by showing that they are similarly fused-linked. No evidence has appeared either for independent assortment or for crossing over of different sex factors with each other. We may speak then of the x-series of alleles, reserving the letter y or z for other pairs or series, if such be found to recombine.

In determining sex-allele relationship of any two stocks, procedure differs according as recessive mutant factors are present or not. If only one stock has a recessive, females of this stock are used in the tests with dominant wild type males of the other stock. If the stocks have different recessives, either or both of the reciprocal crosses may be made. If the stocks are phenotypically similar, a dominant or semi-dominant factor must be introduced into one of them. The semi-dominant body color gene lemon, *le*, has proved very useful in this connection. A cross is made with lemon, and a few F_2 lemon males are mated with females of one of the two stocks. Half of these males should sire diploid sons, recognizable as being heterozygous lemon like the daughters and indicating the sire to be a recombination of lemon with one of the alleles of this stock. Lemon males produced by the daughters may then be used for crossing with females of the other stock in the relationship test.

Lemon has also been used for combining any recessive gene with the sex alleles of a given stock. For example, white eye color, *wh*, was thus combined with the sex alleles of wild type stock 1 from Lancaster, Pa. A white lemon male was crossed with a female of stock 1. White lemon F_2 males from this cross were then mated to stock 1 females, and white stock was extracted from the progeny of those males siring diploid sons. White females of this stock produce wild type diploid sons when bred to stock 1 males and therefore have the same sex alleles.

In like manner fused has been combined with the sex alleles of different stocks, but here it is advisable to test several F_2 fused males because of sex-linkage. Since crossing over is about 10 percent, the expectation is that only one in ten F_2 fused males will sire diploid sons when crossed with females of the given stock. On the other hand, nine in ten F_2 fused males will have the sex allele of the stock originally containing fused.

CROSSES PRODUCING NO FUSED FEMALES

In December 1939 investigations were commenced to determine the relationships in respect to the sex factors of the stocks then being kept in the laboratory. Preliminary tests were made, and stocks were tentatively designated by x alleles. Subsequently (or in some cases simultaneously) crosses were made to determine the linkage of the sex differentiator with fused as a test of allelism in the x series, to exclude the possibility of y or z series. Some of the stocks tested had been derived directly from wild cultures, others from various combinations and with different mutant genes.

The sex factors of a certain stock containing the mutant gene veinless (stock 36-*vl*) were designated xa/xb, and it was shown by crossing that two other stocks, with stubby and with orange and stubby (stocks 35-*sb* and

37-*o.sb*), likewise had these sex alleles. A wild type stock (11-+) and related stocks orange-eyed (stock 11-*o*) and orange carrying fused (stock 11-*o.fu*) proved to have different factors which were therefore designated xc/xd. Factors of wild type stocks 32 and 33 from California proved similar to each other but differed from xa/xb and xc/xd and were therefore designated xe/xf. These two stocks may have been derived from the same wild source. Wild type stock 1 from Lancaster, Pa., which had been bred in the laboratory since 1919, proved to have factors different from the others tested. They were therefore designated xg/xh. Wild type stock 25 from New York City had one factor in common with the xa/xb stocks, giving diploid sons in some of the crosses. No diploid sons were produced in crosses with the other stocks. The sex factors were therefore designated xa/xi.

Different recessive eye colors were combined with the sex alleles of the stocks mentioned above so that diploid males could be identified. Orange (*o*) was retained in the xa/xb stocks, mottled (*mo*) was combined with xc/xd, red (*rd*) with xe/xf, white (*wh*) with xg/xh and cataloup (*c*) with xa-xi. Crosses were made in all cases between recessive females and dominant males. Within stocks or between stocks with the same sex alleles, fraternities sired by 75 males with diploid sons totalled females 2,118, haploid males 1,478, diploid males 411. Six small fraternities, totalling 138 females, 54 haploid males, lacked diploid males, probably as an error of sampling. The diploid males are (411/2,256) 18.2 percent viable relative to the females. The females are (2,256/1,532) 1.47 times as numerous as the haploid males.

There were 160 fraternities sired by 158 males outcrossed to females of the other stocks. These showed that the stocks involved had different sex factors, since no diploid sons were produced. Progenies totalled 8,029 females, 2,901 haploid males. Females are 2.76 times as numerous as their haploid brothers in contrast to the ratio 1.47 found in the two-allele fraternities where one-half of the females are replaced by diploid males or unhatchable eggs.

Crosses of stocks ancestral to some of those recently studied and presumably bearing the same sex alleles have been reported in published literature. Orange-eyed Lancaster females, ancestral to stock xg/xh, were crossed before 1924 to Iowa City males, ancestral to stock xc/xd (WHITING 1940a), giving wild type females 5,409, orange males 3,974. Stock 11-*o* females (xc/xd) were crossed with New York stock 25 males (xa, xi) (BOSTIAN 1935; BISHOP 1937; WHITING 1938), producing wild type females 3,998, orange males 2,685. Stock 11-*o* females (xc/xd) were crossed with stock 32 California males (xe, xf) MAXWELL 1938, WHITING 1938), giving wild type females 8,422, orange males 3,022. The possibility exists that mutations may have occurred in the sex alleles, but they are not such as to produce similarity of those that were formerly dissimilar. Sex ratios in these fraternities are affected by many different conditions. These have been discussed in the publications.

New York stock 25 and cantaloup stock graded to stock 25 (xa/xi) were tested by crossing with xa/xb stocks. Five cantaloup females produced females 216, haploid males 55, while twelve produced females 317, haploid males

241, diploid males 79. Nine xa/xb females produced females 383, haploid males 171, while nine produced females 341, haploid males 282, diploid males 70. The 21 females with diploid sons produced (658/523) 1.26 times as many daughters as haploid sons, while the 14 females with no diploid sons produced (599/226) 2.64 times as many. This doubled ratio along with lack of diploid sons is good evidence that the two stocks have only one allele in common.

CROSSES PRODUCING FUSED FEMALES

Many of the fraternities included in the data discussed above involved the gene fused but in such a way that it was of no significance as regards sex allele relationship. Only when matings produce fused daughters, when the female is heterozygous and the male fused (+/*fu*×*fu*), are the data for fused significant. The ratio of fused among the females then indicates whether three or only two alleles were involved in the mating even though the presence of diploid males cannot with certainty be established because of lack of a recessive differentiator for which the mother was homozygous. However, the distribution of wing microchaetae as an index of cell size may be noted and the presence of the larger-celled diploid males determined. The character fused also differs markedly in the diploid males, being much more extreme and similar to that in the females. Both traits, cell size and fused antennal length, show overlapping between haploid and diploid males and are evidently subject to modifiers.

Difficulties have been experienced in working with fused, not only because of its female sterility which makes it impossible to maintain homozygous stock, but even more because the malformation of appendages renders it difficult and often impossible to obtain matings of the males. The character is subject to genetic modifiers which differ markedly in different stocks. When the gene fused is graded to certain stocks, the segregates may be exceedingly malformed, but in other stocks the appendages of the fused males may be relatively long and straight.

Another source of difficulty in obtaining matings from the relatively weak fused males is the resistance of certain females to mating. There appears to be a genetic factor affecting this reaction which is much more pronounced in some stocks than in others.

ALLELISM OF NINE SEX-DIFFERENTIATING FACTORS

The crosses summarized above show that the five groups of stocks contain different sex differentiators, except that the New York stock 25 (xa/xi) has one factor in common with the laboratory stocks with sex alleles xa/xb. Allelism of these factors was shown by testing their linkage with the gene fused. Fused had been maintained by grading to stock 11-*o*, the sex factors of which were tentatively called xc/xd. Fused was then combined with the sex differentiators of the xa/xb stocks and subsequently with those of the others. As tested in each combination, fused proved to be sex-linked, or, in other words, the sex differentiators proved to be similarly fused-linked and

therefore in all probability allelic. The diploid offspring of the separate fraternities (summarized in table 2) show that when fused is in low ratio among the females, it is high among the males, and the reverse. The haploid male offspring show no such relationship, but ratio of fused fluctuates about equally with non-fused or drops low when there are certain factors decreasing its relative viability. Thus from veinless mothers the haploid sons were 164 veinless, 107 veinless fused, but from all mothers marked by recessive eye colors the haploid sons were non-fused 899, fused 864, showing no deleterious effect of these genes in connection with fused.

In general in those fraternities from mothers with no recessive marker for separating their haploid sons from the diploid, there was deficiency of fused among total males when there was excess among the females, such males totalling non-fused 402, fused 306, and there was excess of fused among total

TABLE 2

Data for crossovers between x and fu in stocks with different sex alleles.

SEX ALLELES	FEMALES						DIPLOID MALES						TOTAL		
	FUSED LOW			FUSED HIGH			FUSED LOW			FUSED HIGH			C.O.	TOTAL	% C.O.
	+	*fu*	%*fu*	+	*fu*	%+	+	*fu*	%*fu*	+	*fu*	%+			
xa/xb	723	81	10.1	57	431	11.6				3	16	15.8	141	1311	10.7
xc/xd	58	11	15.9	1	29	3.3				1	15	6.2	13	115	11.3
xe/xf	928	152	14.1	21	149	12.4	9	2	18.2	16	65	19.7	191	1342	14.3
xg/xh	651	78	10.7	21	233	8.2	30	5	14.3	4	72	5.3	108	1094	9.8
xa/xi	420	42	9.1	20	88	18.5	40	9	18.2	15	179	7.7	86	813	10.6
Total	2780	364	11.5	120	930	11.4	79	16	16.8	39	347	10.1	539	4675	11.5

males when there was deficiency among the females, such males totalling non-fused 870, fused 1,191. The presence of diploid sons is thus evidenced.

As explained above, when the gene fused was being transferred from one stock to another in order to combine it with the sex alleles of different stocks, the F_2 fused males from the crosses were tested by crossing them to the females of the stock into which it was desired to introduce the gene fused. Of 51 such males tested, six sired diploid sons. These males are the ten percent crossovers expected.

Fused-linkage of x in the different stocks

Table 2 summarizes the data from the two-allele fraternities on recombination of x and *fu*. These are given separately according to sex alleles, for females and for diploid males and for the two kinds of fraternities phenotypically different as to proportion of fused. The summaries as to sex and for the two kinds of fraternities indicate by a χ^2 test no significant differences in percentage of crossovers. Totals with respect to the stocks carrying the different sex alleles show a higher proportion of crossovers in xe/xf, while the four other stocks deviate more or less below the mean and are not significantly different from each other. If they be summarized as one population (348 crossovers among

3,333, 10.5 percent), the chance that xe/xf might belong to this population is infinitesimal.

Previously found differences in strength of linkage between sex alleles and fused have been summarized and discussed elsewhere (WHITING 1940a). There was from 8.6 to 17.6 percent crossing over.

Three-allele fraternities including fused females

In crosses of females heterozygous for fused to fused males with a third sex allele, non-fused and fused should occur with equal frequency both in females and in haploid males except for viability difference resulting in deficiency of fused. No diploid males are produced, and female ratio is correspondingly high. As in the case of two-allele crosses, the ideal mating should involve a genic

TABLE 3

Three-allele crosses (+/fu×fu) producing fused females.

MOTHERS		FATHERS		DAUGHTERS		SONS	
SEX ALLELES	NUMBER	SEX ALLELES	NUMBER	NON-FUSED	FUSED	NON-FUSED	FUSED
xa/xb	15	xc, xd	9	365	283	88	55
xc/xd	7	xa, xb	4	101	79	29	32
xe/xf	16	xa, xb	15	318	293	92	110
xe/xf*	5	xa, xb	5	128	112	73	67
xg/xh*	7	xa, xb	7	182	207	111	122
xe/xf*	7	xg, xh	7	267	241	107	110
xg/xh*	3	xe, xf	3	140	136	55	58
xe/xf*	6	xa, xi	6	266	230	157	138
xg/xh*	1	xa, xi	1	75	73	43	33
xa/xi*	6	xg, xh	6	275	283	115	123
Total	73		63	2117	1937	870	848

* These females were homozygous for some recessive for which the males possessed the dominant allele. All sons were recessive, indicating absence of diploid males.

difference for which the female is recessive, which may serve as a check on the absence of diploid males, but if this be lacking the ratio of fused among the females is usually adequate to separate two-allele from three-allele fraternities in miscellaneous material.

Table 3 shows the results of crosses made between the different inbred stocks in each of which the sex differentiating factors have been shown to be linked with fused. Because of technical difficulties resulting from malformation of fused combined with xc/xd, this material was tested with xa/xb only. However, tests of xc/xd not involving fused show that the sex differentiating factors xc and xd are different from xe, xf, xg, xh, and xi, as well as from xa and xb.

Among the two-allele fraternities those showing an excess of fused females and those showing a deficiency were regarded as genotypically different. The

excess or the deficiency was always so extreme that there was never any question as to the proper classification, unless the fraternity was exceedingly small.

In the 73 fraternities of table 3, non-fused and fused daughters average not far from equality, individual fraternities fluctuating with no marked cases of deviation and the most extreme deviates being the smallest. In order to show further that the fraternities produced by crossing the different inbred stocks make up a population fundamentally different from those resulting from the closecrosses, these fraternities were separated according to excess or deficiency of fused among the females. Each of six fraternities showed equality, totalling 88 non-fused, 88 fused. Each of 44 fraternities had deficiency of fused females totalling 1,532 non-fused, 1,202 fused (44 percent fused), while each of 23 had excess of fused totalling 497 non-fused, 647 fused (43.4 percent non-fused). There is a barely significant deviation of fused below equality among the total offspring in the 73 outcross fraternities, (1,937/4,054) 47.8 percent fused among females and (848/1,718) 49.3 percent fused among males. The data are in complete agreement with the 1:1 ratio of the female-producing zygotes expected.

LOCATION OF X IN THE LINKAGE MAP

Linkage of genes to the left of x

Linkage of the genes reduced (*r*) and glass (*gl*) has been shown (WHITING and BENKERT 1934) with about 13 percent crossing over. New data from *r/gl* females are males +29, *r* 298, *gl* 260, *r gl* 24, total 53/611, 8.7 percent crossovers and from +/*r gl* females, males +1,032, *r* 105, *gl* 149, *r gl* 911, total (254/2,197) 11.5 percent crossovers. Semi-reduced (r^s) an allele of reduced, was also crossed with glass, giving F_2 males, +59, r^s 186, *gl* 228, r^s *gl* 53, total (112/526) 21.3 percent crossovers.

The gene speckled (*Sk*) puts small red specks in white eyes and requires white (*wh*) as a differentiator. Among 1,236 white F_2 males from +/*Sk wh* there were 583 speckled, 47 percent, indicating independent segregation.

From *r gl/Sk wh* females there were bred 4,297 males, +955, *r* 152, *gl* 147, *r gl* 911, *wh* 133, *wh r* 149, *wh gl* 132, *wh r gl* 919, *wh Sk* 793, *wh Sk r* 6. Since *Sk* needs *wh* as a differentiator but is masked by *gl*, only the 1,081 white, non-glass constitute an effective count for the three loci. The genes with intervals may then be arranged *Sk*−12.9−*r*−14.3−*gl* with a coincidence of 31.6 percent, (6×1,081/139×155). Total crossovers between reduced and glass in this experiment are (586/4,297) 13.6 percent.

In a count of 1,432 males from *wh Sk sb/r gl le* females, carried out by MAGNHILD TORVIK GREB and RAYMOND J. GREB, summary with respect to reduced and glass is +609, *r* 124, *gl* 191, *r gl* 508 (315/1,432) 22 percent crossovers. The 419 white non-glass in this count summarized for $\frac{\text{Sk}\ (1)\ +\ (2)\ +}{+\qquad r\qquad gl}$ were *wh Sk* 291 (non-crossovers), *wh* 61 (region 1 crossovers), *wh r* 40 (region 2),

wh Sk r 27 (regions 1, 2). Crossing over is 21 percent for region 1, 16 percent for region 2, and there is no interference.

Linkage of genes to the right of x

A very long linkage group lies to the right of x. We are here concerned only with the relationships of fused and stubby, since these are near enough to show sex linkage.

Abnormal antennae (*sb*^{a}), an allele of stubby (*sb*), was crossed with fused, and three small F_2 fraternities were bred totalling males +23, *fu* 68, *sb*^{a} 60, *fu sb*^{a} 17, (40/168) 24 percent crossovers. The double mutant males had flagellum of antennae reduced to a small knob and legs very misshapen. Nevertheless a single mating was obtained of a fused abnormal male with a wild type female. F_2 males were +697, *fu* 115, *sb*^{a} 202, *fu sb*^{a} 416, (317/1,430) 22.2 percent crossovers.

Two F_2 fraternities from stock 35-*sb* females by a single 11-*o fu* male totalled (disregarding orange) 746 males, +118, *fu* 301, *sb* 275, *fu sb* 52 (170/746) 22.8 percent crossovers. The *fu sb* males were similar to the *fu sb*^{a} obtained in the preceding experiment.

HAGER (1941) reported counts from *sb/fu* females totalling 1,301 males, +179, *fu* 507, *sb* 479, *fu sb* 136, 24.2 percent crossovers.

Sex linkage of fused and stubby

The strength of the sex linkage of fused has been shown to vary from 8.6 percent (SPEICHER) to 17.6 percent (BOSTIAN) crossing over (WHITING 1940a).

When the mutant type abnormal antennae (*sb*^{a}) was being isolated as a stock, fraternities occurred suggesting sex-linkage. Heterozygous females were being crossed with abnormal males. Of 21 such fraternities each of eight had wild type and abnormal females in practically equal numbers, females +124, *sb*^{a} 130, males +127, *sb*^{a} 136. The remaining thirteen fraternities showed a marked deficiency of abnormal females which were in each case approximately only half as numerous as wild type, females +371, *sb*^{a} 157, males +225, *sb*^{a} 254. Presumably three-allele crosses and one of the possible types of two-allele crosses were occurring here in this material that contained more than two of the sex factors.

Subsequently a series of experiments was carried out with stubby which had been combined with the xa/xb sex alleles so that all crosses were of the two-allele type. In these experiments either veinless females heterozygous for stubby were crossed with non-veinless stubby males or orange females heterozygous for stubby were crossed with non-orange stubby males. Fraternities from 12 stubby males showing excess of non-stubby females totalled females non-*sb* 288, *sb* 162, diploid males non-*sb* 10, *sb* 54, haploid males non-*sb* 145, *sb* 123. Fraternities from 13 stubby males showing excess of stubby females totalled females non-*sb* 87, *sb* 239, diploid males non-*sb* 34, *sb* 8, haploid males non-*sb* 77, *sb* 70. By the square root of prod-

ucts method these data give for the females 31.2 percent crossing over and for the diploid males 17.3 percent. The figure for the females is more nearly accurate because of larger numbers involved. In HAGER'S (1941) work with the xa/sb material orange females heterozygous for stubby were crossed with stubby males. The zygous offspring were in one group females +370, *sb* 228, males +10, *sb* 32, and in the other group females +642, *sb* 1,132, males +70, *sb* 57. By the square root of products method crossing over is 37.5 percent among the females and 33.6 percent among the diploid males.

Linkage across x

Counts of 325 white males from +/*wh Sk sb* females were *wh* 111, *wh Sk* 70, *wh sb* 69, *wh Sk sb* 75. The white non-glass from *wh Sk sb/r gl le* summarized for *Sk* and *sb* were *wh* 54, *wh Sk* 159, *wh sb* 47, *wh Sk sb* 159. (On account of linkage with glass about three-fourths of these non-glass are Speckled.) Speckled and stubby appear to segregate independently.

F_2 males from *wh Sk* females crossed with fused males were +892, *fu* 672, *wh* 458, *wh Sk* 415, *wh fu* 367, *wh Sk fu* 237 and F_2 males from *wh Sk* females crossed with white fused males were *wh* 369, *wh Sk* 374, *wh fu* 289, *wh Sk fu* 232. Among 2,741 white-eyed there are 1,445 recombinations indicating no linkage between speckled and fused.

Stubby shows very slight, if any, linkage with reduced or with glass. From *r*/*sb*^{a} females there were 653 males, +166, *r* 164, *sb*^{a} 167, *r sb*^{a} 156, and from *gl*/*sb*^{a} females there were 616 males, +159, *gl* 172, *sb*^{a} 141, *gl sb*^{a} 144. In each case the total non-crossovers are insignificantly higher than the recombinations.

Two experiments, in one of which 733 males were bred from *sb*^{a}/*r gl* females and in one of which 1,432 males were bred from *wh Sk sb/r gl le* females, were summarized for $\frac{+ \quad (1) \quad + \quad (2) \quad sb \text{ or } sb^a}{r \qquad gl \qquad +}$ (table 4). It may be seen from the table that for each pair of alternative classes the class containing reduced is somewhat lower. Each of two types of region 2 singles is respectively lower than the comparable (for *r*) type of non-crossovers and, the same holds for the doubles as compared with region 1 singles. Total recombinations between *gl* and *sb* are 1,013/2,165, 46.8 percent, showing a deviation from independent assortment to be expected in less than one percent of cases and indicating loose linkage. Crossovers in region 1 are 18.4 percent.

Recent data from *r*/*fu* females include 539 males, +170, *r* 178, *fu* 120, *r fu* 71, and from *r*^{s}/*fu* females 592 males, +159, *r*^{s} 170, *fu* 136, *r*^{s} *fu* 117, indicating linkage (517/1,131) 45.7 percent crossovers. Published data (WHITING and BENKERT 1934) on 3,196 sons of +/*r fu* females and 2,844 of *r*/*fu* showed 45.5 percent recombinations among the non-fused which was taken as the best estimate to avoid viability effects.

TABLE 4

Sons of unmated females, $\frac{+}{r}$ (1) —18.4— $\frac{+}{gl}$ (2) —46.8— $\frac{sb \text{ (or } sb^a)}{+}$.

ALTERNATIVE CLASSES		TOTALS	
sb (or sb^a)	496		
r gl	445	non-crossovers	941
gl	127		
r sb (or *r* sb^a)	84	region 1 c.o.	211
+	477		
r gl sb (or *r gl* sb^a)	348	region 2 c.o.	825
gl sb (or *gl* sb^a)	105		
r	83	doubles	188
		Total	2165

Linkage of fused and glass. Sex-linkage of glass (?). Inviability of sperm (?) in +/gl, wa/wa, gz/gz and gz^s/gz^s *females*

Among 547 sons of *fu/gl* females (WHITING and BENKERT 1934) there were 41.7 percent recombinations among the non-fused, suggesting that glass is nearer to fused than is reduced. Recent experiments bear out this conclusion. In these experiments the attempt was made to test sex-linkage of glass, but this was proved impracticable because females heterozygous for glass produce very few daughters. Glass males are highly fertile, siring normal numbers of daughters when bred to non-glass females. Glass females are sterile, probably became their feet are weak and they are consequently unable to obtain nourishment by stinging and feeding upon the caterpillars. Females heterozygous for glass feed normally and produce abundance of male offspring. Forty-six heterozygotes of which at least 27 were mated and 14 exposed to males produced haploid sons, +1,472, *gl* 1,455 but only 56 daughters and seven diploid sons. Of the daughters, at least 12 were known from their genetic markers to be impaternate, and consequently there were not more than 51 offspring sired by the males.

A similar situation obtains when the females are heterozygous both for glass and for fused. From 22 *fu/gl* females, of which at least 11 mated, there were only 26 daughters, while sons were +317, *gl* 561, *fu* 418, *gl fu* 176. From 38 *+/gl fu* females, of which at least 19 mated, there were only 59 daughters, while sons were +904, *gl* 575, *fu* 384, *gl fu* 600. Recombinations between *gl* and *fu* total (1,452/3,935) 37 percent in these recent tests.

In connection with the failure of *+/gl* females to produce more than a few daughters, it may be noted that the same applies to wavy (*wa*) wings females and to glaze (*gz*) and shiny (gz^s) an allele of glaze. Wavy and glaze females are weak, producing relatively few offspring, and among these, daughters are very infrequent. Shiny females, like heterozygous glass, produce an abundance of

sons. Males of all three mutant types are highly fertile so that something evidently prevents the eggs and sperm from getting together. Comparison may here be made with conditions governing infertility of glossy females in Drosophila which, according to ANDERSON and OLIVER (1942), "seems to be associated with inviability of sperm in the females rather than with egg disturbance."

Genotypic differences not associated with any visible trait may likewise affect viability of sperm in the female and thus be a factor influencing sex ratio in Habrobracon. Unpublished data collected in 1933 by B. R. SPEICHER suggest this. At that time DR. SPEICHER made an attempt to test sex-linkage of glass. Offspring from several heterozygous females by glass males totalled 785 females, 2,187 males, showing a much higher female ratio than that later obtained by the author. Furthermore some of the crosses made by DR. SPEICHER were of fathers to daughters in which female ratio would be lowered because of age of sire and reduced sperm supply. There were no clear cut ratios with respect to glass among the females. When glass was below 50 percent among the females (179/448=40.0 percent) it was also low among the males (622/1,314=43 percent), and when it was above 50 percent among the females (191/337=56.7 percent) it was raised among the males (434/873=49.7 percent). Inviable pupae were (226/4,990) 4.6 percent in the former group and (135/1,345) 10 percent in the latter. No evidence is therefore available for sex-linkage of glass, which may be very loose or may be masked by a third allele and by viability differences in DR. SPEICHER's data.

The linkage map

A linkage map of regions near x with approximate intervals may be constructed as follows:—

Sk —13— *r* —15— *gl* x —10— *fu* —24— *sb*

---------37---------

---------35----------

ANDERSON and WHITING (1939) reported a "cantaloup" group of 14 loci with map distance 187 units and a "red" group of five loci with map distance 74 units. CLARK (1942) reported data from six loci scattered along about 70 units of the cantaloup group and indicating no interference across one of these loci, lemon. CLARK suggested that the spindle fiber might be near lemon. The cantaloup group has proved to be linked to the right of stubby, while the red group is linked to the right of the cantaloup group. The series of sex alleles is probably approximately in the middle of the left arm which appears to be somewhat shorter in crossover units than the right, according to data now available.

DISCUSSION

Applicability of complementary scheme to other haplo-diploid cases

Various writers have questioned the applicability of the principles of sex determination advanced for Habrobracon to the Hymenoptera in general and to

the other groups of invertebrates with haploid males and have preferred to regard Habrobracon as a special case. Diploid males have been shown in two other species of Habrobracon (SPEICHER and SPEICHER 1940; INABA 1939), but to the author's knowledge no one has yet applied such breeding and observational tests as might reveal them elsewhere. However, DOZORCEVA (1936) found a sex-linked trait in *Pteromalus puparum*, belonging to the Chalcidoidea, a different superfamily of the Hymenoptera. GREENSHIELDS (1939) interpreted DOZORCEVA's data as being in disagreement with the complementary theory, but it is clear from GREENSHIELDS' review that he misunderstood the theory (WHITING 1940b). DOZORCEVA showed three different kinds of fraternities with respect to the occurrence among the females of the mutant sex-linked trait—namely, those with marked excess, those with marked deficiency indicating two-allele crosses, and those with equality indicating three-allele crosses. Her data were inadequate to prove whether differential maturation, multiple factors, or multiple alleles were at play, and she had no fraternities from recessive mothers which might have revealed diploid males.

It has been thought that the complementary theory might not hold in all cases because of evidence from thelytokous species or races. This point of view has been presented in an excellent paper by SMITH (1941), who gives a very thorough discussion of the literature and whose cytological work appears to be of the highest order. Thelytokous forms produce females by indefinite parthenogenesis and show cytologically a variety of conditions ranging from complete apomeiosis to normal meiosis with subsequent chromosome doubling either by interkinetic separation of chromatids or by fusion of cleavage nuclei. The latter would result in complete homozygosis, while apomeiosis would duplicate the genotype of the mother. Thelytoky, which has certain advantages for rapid dispersal through new territory but lacks the benefits usually associated with amphimixis, is polyphyletic in the Hymenoptera and secondary to the well-established condition with haploid males. The latter, known as arrhenotoky, has been shown to be characteristic of the Hymenoptera from the most primitive sawflies to the ants and bees and must have been attained very early in the evolutionary history of the order.

If single meiotic division accompanies thelytoky, heterozygosis of x might be maintained if this division were equational, or "homozygous" females might be produced if x were duplicated either by translocation or by unequal crossing over so that xa/xb + 2A would become xa xb/xa xb + 2A. Theoretically, haploid females xa xb+1A would then be possible. That such have not been found is not surprising, however, since thelytokous forms tend to become polyploid rather than haploid, as in Artemia, Trichoniscus, Solenobia, and many others. Thelytokous races are believed to revert to arrhenotokous and thus again enjoy the advantages of amphimixis, but presumably this reversion would be rendered difficult or impossible if apomeiosis had become firmly established or if many recessive lethals were accumulated.

DARLINGTON (1937), before the multiple allele theory was advanced, discussed the XY mechanism of Habrobracon. He pointed out the antiquity and

perfection of haplo-diploid system found in so many groups of organisms, but he held that "the particular XY mechanism shows some signs of newness." It seemed likely to him that the differential chromosome segment "never could develop except in the direction of inertness, and the fact that the two kinds of males do not differ in any observable respect bears out this expectation." A point seemingly overlooked by DARLINGTON was the implications of haploid parthenogenesis for natural selection (WHITING 1935). "Both X and Y chromosomes contain dominant as well as recessive factors and are equally subject to natural selection. They cannot, therefore, degenerate by the accumulation of recessive lethals. Were it not for parthenogenesis, the complementary scheme might shift over into one of simple dominance, the crossing of a heterozygote with a recessive as in other forms."

Sex differentiation and the nature of x

Not only is sex dimorphism very great in many groups of Hymenoptera, but along with this divergence in structure go great differences in reaction, reaching a culmination in the social groups in which the females assume the entire care of the colonies while the social instincts of the males, drones, show no phenotypic advance. Yet the germ plasm of the drones does not differ from that of the females in any way except that the one is normally haploid, the other diploid. Now if diploid males occur in the social forms and if these are nevertheless drones, then it is not number of chromosome sets that makes the difference but rather azygosis (or homozygosis) versus heterozygosis, the latter with its complementary masking of recessive male traits both structural and "psychic." Here it should be mentioned that in Habrobracon the diploid males are just as male both in reactions and in structure as are the haploid. The problem then centers about two questions—(1) whether we are dealing with a single difference segregating as a unit in oögenesis or with many differences and (2) if the former be true whether there be a single alternative pair with some sort of differential maturation or selective fertilization to avoid reproductive waste or a series of alleles. Reproductive economy is assured by multiple factors, by differential maturation, or by multiple alleles. Since the last has been demonstrated to govern the multiplex sex differences of Habrobracon, there would seem to be no reason why the principle should not be extended.

The nature and evolution of the sex factor x, the differential segment, may now be considered. Mutant genes have been shown to cross over both to the left and to the right of x, hence x is interstitial. Is there crossing over within x? If this were so, x would be inconstant, xa would not remain distinct from xb, or xb from xc, etc. The work of HORN (1943) demonstrates that F_2 males segregating from crosses of xa/xb with xe/xf stocks are still xa, xb, xe, or xf, they all sire diploid sons, and those that were xa or xb sire diploid sons by xa/xb females and not by xe/xf females and the reverse (as shown by linkage with fused). It may be concluded therefore that there is no crossing over within x; or perhaps the rare "mutations" of x, if such actually occur, may be crossovers.

The complementary nature of a series of multiple alleles may now be considered in an attempt to answer STURTEVANT'S (1941) question as to how one may "picture the action of a third allele" or "of a still larger series of mutually complementary alleles." Were we dealing with a single trait, we might assume a number of recessive genes each resulting in a similar phenotype and each bound by failure of crossing over with the dominant alleles to all the others. For this allelic series any heterozygote would be dominant, any homozygote (or azygote) recessive. Now imagine duplicate genes governing all the trait differences between the sexes and similarly distributed along the differential segment. The result would be the multiple allelic series required. It is not necessary, however, to suppose that in the evolution of this series all the genic differences governing any one trait would have to be duplicates. Each allele might be built up from a few major genic differences and the details perfected and phenotypically equalized by minor genes and modifiers.

Sex genes tend toward maleness or toward femaleness with respect to the general genome of a species so that any given intersex gene for either sex is a supersex gene for the other. However, if sex differentiation is already normally pronounced, supersexuality is less obvious phenotypically than intersexuality. The gene gynoid in Habrobracon, for example, causes males to be intersexual but has no effect on females, and a great range of intersexes occurs in Lymantria after race crossing but no supersexes.

Having attained then a certain degree of sex disparity, considerable genic difference might exist between the alleles without becoming apparent. If genic differentiation is very wide in the Hymenoptera, there may be considerable subliminal genic deviation among the alleles. This should be further investigated by statistical study of the effects of the different alleles on sex differences. Data on counts of antennal segments, collected in 1922, show that in the Lancaster stock antennae of males were longer and of females shorter than in the Iowa City stock. Difference in number of segments was 0.715 ± 0.040 S. E. between the females and 0.343 ± 0.037 S. E. between the males and in the opposite direction. The antennae of the Lancaster stock were more differentiated sexually, while those of the Iowa City stock indicated a slight step toward intersexuality. It now seems reasonable to suppose that the genes concerned were located in the differential segment, for had they been elsewhere they would have segregated independently of sex, and the stock showing the longer male antennae would have averaged longer antennae in the females also.

In Drosophila sex may be regarded as determined by a pair of allelic factors, X and Y, segregating from each other in spermatogenesis. The fact that crossing over occurs between the two X chromosomes of the female furnishes the possibility of analyzing racial differences in genes which differentiate sex, but analysis has been made chiefly by the use of translocations. The sex differentiator of Drosophila has proved to by polygenic. The differential segment of Habrobracon will probably prove so as well.

SUMMARY

The multiple allele theory of sex determination in Habrobracon has been exhaustively tested. Laboratory stocks have been analyzed for sex factors. Technique consisted in selecting one stock, designating its sex alleles as xa/xb, and introducing into it a recessive gene. If all significantly large fraternities from crosses of recessive females of this stock to dominant males of an unknown stock contained dominant males (diploid), as well as the recessive males (haploid) and dominant females (diploid), the P_1 male stock was considered to have the same sex alleles, xa/xb. If no dominant males (diploid) appeared in any of the fraternities, the P_1 male stock was regarded as having different alleles—for example, xc/xd. If only one-half the fraternities included dominant males, the P_1 male stock was considered to have one allele in common with xa/xb—for example, xa/xi. Recessive genes were introduced into each new stock as it was analyzed for sex factors, and "tester" stocks were made up for future use.

Nine factors are thus far known in the series, and the tester stocks are designated xa/xb, xc/xd, xe/xf, xg/xh and xa/xi.

In three-allele crosses (xa/xb×xc) the diploid heterozygous daughters (xa/xc and xb/xc) are approximately twice as numerous as the haploid, azygous sons (xa and xb). This ratio depends upon proportion of eggs fertilized. In two-allele crosses (xa/xb×xa) the additional class, diploid homozygous sons (xa/xa), replaces one-half of the females but such males are of low viability, many failing to hatch, so that ratio of diploid males to females is lowered.

Evidence of allelism of these sex factors is furnished by the fact that they are all closely linked with the gene fused. When mothers were heterozygous for fused and fathers fused, linkage was shown by the offspring from all two-allele crosses, ratio approximating nine wild type to one fused among the females and one wild type to nine fused among the diploid males, or the reverse, according as fused is associated with a different or with the same sex allele in the mother as in the father. In three-allele crosses linkage is masked because both non-crossovers and crossovers are female and non-separable. Fused and non-fused females are therefore numerically equal.

Lack of diploid sons and equality of fused and non-fused daughters from crosses of stocks tentatively designated as having different sex alleles in the x series proves the sex factors to be different, but their linkage with fused indicates their allelism.

Linkage data show three genes to the left of x, but sex-linkage of glass, the nearest of these, could not be tested because heterozygous females produce very few daughters. Stubby is loosely sex-linked and about twenty-four units beyond fused which is ten units to the right of x.

No evidence has yet appeared contrary to extension of the multiple allele theory to Hymenoptera in general or to other invertebrates with haploid males.

The multiple alleles are regarded as differential chromosome segments which have been built up in the early evolution of the Hymenoptera, are necessarily

associated with haploid parthenogenesis and consist of many genes determining the numerous sex differences, structural, functional and behavioristic, characterizing Hymenoptera. These genes have, in the aggregate, duplicate effects such that all haploids or homozygous diploids are similar and male, but combinations of any two different alleles result in females (heterozygous dominants), likewise all similar.

With n alleles in the series there should be n genetically different haploid males, n corresponding homozygous diploid males and $(n^2-n)/2$ possible heterozygous female-producing combinations.

LITERATURE CITED

ANDERSON, R. C., and C. P. OLIVER, 1942 Effect of double matings on the fertility of a mutant of *Drosophila melanogaster*. Anat. Rec. **84**: 60.

ANDERSON, R. L., and P. W. WHITING, 1939 Present status of linkage groups in Habrobracon. Genetics **24**: 65.

BISHOP, D. W., 1937 Induction of dominant lethal effects by X-radiation in Habrobracon. Genetics **22**: 452–456.

BOSTIAN, C. H., 1935 Biparental males and hatchability of eggs in Habrobracon. Genetics **20**: 280–285.

1939 Multiple alleles and sex determination in Habrobracon. Genetics **24**: 770–776.

CLARK, A. M., 1942 Linkage relations of six factors in Habrobracon. J. Hered. **33**: 78–80.

DARLINGTON, C. D., 1937 Recent advances in cytology. 2nd ed. Philadelphia: P. Blakiston's Son and Co.

DOZORCEVA, R. L., 1936 Sex-linked heredity in *Pteromalus puparum*. C. R. (Doklady) Acad. Sci. URSS 3 (12), **7** (102): 335–338.

GREENSHIELDS, F., 1939 Whiting's hypothesis and Pteromalus: a critique of Dozorceva's (1936) study. Amer. Nat. **73**: 89–91.

HAGER, R. P., 1941 Sex-linkage of stubby (*sb*) in Habrobracon. Biol. Bull. **81**: 298–299.

HORN, ANNABELLE B., Proof for multiple allelism of sex-differentiating factors in Habrobracon. Amer. Nat. (in press).

INABA, FUMIE, 1939 Diploid males and triploid females of the parasitic wasp *Habrobracon pectinophorae* Watanabe. Cytologia **9**: 517–523.

MAXWELL, JANE, 1938 Inactivation of sperm by X-radiation in Habrobracon. Biol. Bull. **74**: 253–255.

SMITH, S. G., 1941 A new form of spruce sawfly identified by means of its cytology and parthenogenesis. Sci. Agric. **21** (5): 245–305.

SNELL, G. D., 1935 The determination of sex in Habrobracon. Proc. Nat. Acad. Sci. **21**: 446–453.

SPEICHER, B. R., and KATHRYN G. SPEICHER, 1940 The occurrence of diploid males in *Habrobracon brevicornis*. Amer. Nat. **74**: 379–382.

STURTEVANT, A. H., 1941 Physiological aspects of genetics. Annu. Rev. Physiol. **3**: 41–56.

WHITING, P. W., 1933 Selective fertilization and sex-determination in Hymenoptera. Science **78**: 537–538.

1935 Sex determination in bees and wasps. J. Hered. **26**: 263–278.

1938 The induction of dominant and recessive lethals by radiation in Habrobracon. Genetics **23**: 562–572.

1939 Sex determination and reproductive economy in Habrobracon. Genetics **24**: 110–111.

1940a Multiple alleles in sex determination of Habrobracon. J. Morph. **66**: 323–355.

1940b Sex-linkage in Pteromalus. Amer. Nat. **74**: 377–379.

1941 Investigations on genetics and sex determination in the parasitic wasp Habrobracon. Yearb. Amer. Phil. Soc. **1940**, 274–276.

WHITING, P. W., and LYSBETH HAMILTON BENKERT, 1934 Azygotic ratios in Habrobracon. Genetics **19**: 237–267.

INCREASED MEIOTIC IRREGULARITY ACCOMPANYING INBREEDING IN DACTYLIS GLOMERATA L.[1]

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VARIATIONS in chromosomal association and behavior during meiosis were found by MYERS and HILL (1940, 1942) among plants of *Dactylis glomerata*. Cytogenetically the plants of this species behave as autotetraploids (x=7). Among replicated clones from nine of the plants studied by MYERS and HILL (1942), MYERS (1943b) found that the differences in chiasma frequency, average number of quadrivalents per sporocyte, percentage of metaphase I sporocytes with univalents, percentage of anaphase I sporocytes with lagging and dividing chromosomes, and percentage of quartets with micronuclei, all of which had been observed in duplicate collections from the greenhouse, persisted in three replications and through two years in the field. It was presumed that the differences could be attributed to differences in genes, chromosomal differentiation, or both. For the validity of this hypothesis the progeny test provides critical evidence. In studies of first inbred generation progenies of eight of the clones, a previously unreported effect of inbreeding upon chromosomal behavior was encountered. An abstract of these results has been published (MYERS 1943a).

MATERIALS AND METHODS

Plants of the first inbred generation (from selfed seed) from the clones reported by MYERS (1943b) were used in these investigations. The inbred progenies were space-planted in the field in rows adjacent to their respective parental clones. Microsporocyte material was collected in 1940 from the plants of six progenies—namely, OG 2 (11), OG 6 (12), OG 7 (20), OG 42 (5), OG 48 (28), and OG 48 (92), and in 1941 from the plants of two progenies—namely, OG 2 (3) and OG 48 (48). All collections in each year were made on a single day to avoid introducing into the results variation due to dates of collection. The collections from the parental clones (MYERS 1943b) were also made on the same day. For the comparison of inbred progeny with parent, only data collected in the same year were used.

All material was fixed in acetic-alcohol and stored in the fixing solution at low temperature in a household refrigerator until studied. All data were obtained from fresh acetocarmine smear slides. In order to eliminate variation due

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to individual differences in interpretation, all meiotic data were collected by the senior author.

EXPERIMENTAL RESULTS

Chiasma frequency

The number of chiasmata per chromosome was determined from an average of 21 diakinesis sporocytes for each plant of the two inbred progenies from which material was collected in 1941 (Table 1). The class interval of 0.08, used in Table 1, is two times the standard error of the difference for a single determination calculated from the error variance obtained for this character in studies of the replicated parental clones (MYERS 1943b). Since the inbred plants were grown in rows adjacent to their parents and were handled in a similar manner, it is expected that their means were distributed with the same error variance as the means of the clones. It seems legitimate, therefore, to

TABLE 1

Average number of half chiasmata per chromosome in two parental clones of Dactylis glomerata and their respective first inbred generation (I_1) progenies.

FAMILY NUMBER	AVERAGE NO. OF ½ x-ta PER CHROMOSOME		NUMBER OF I_1 PLANTS WITH INDICATED NUMBER OF ½ x-ta PER CHROMOSOME						
	PARENT	I_1	1.35* to 1.43	1.44 to 1.51	1.52 to 1.59	1.60 to 1.67	1.68 to 1.75	1.76 to 1.83	1.84 to 1.91
OG 2 (3)	1.78	1.71	1	—	2	4	5	6	2
OG 48 (48)	1.77	1.82	—	—	—	—	—	5	1

* Class interval of 0.08 equals 2×S.E. of the difference for a single determination. (Based on error variance of the parental clones.)

consider any two inbred plants significantly different in chiasma frequency if they differ by more than one class interval in Table 1. Among 20 plants in the progeny of OG 2 (3) it is apparent that segregation occurred for average number of chiasmata. The extreme plants had 1.35 and 1.90 half chiasmata per chromosome, respectively. The average chiasma frequency of the inbreds was 1.71 compared to 1.78 for the parent. The difference of 0.07 is significant; $0.05 > P > 0.01$.

In the progeny of OG 48 (48), the six plants ranged from 1.80 to 1.88. Since the difference between the extreme plants is equal to exactly twice the standard error of the difference, it cannot be concluded from these data that segregation occurred in this progeny. The average chiasma frequency of the inbred plants exceeded that of the parent by 0.05, but the difference is not significant.

The two parents had nearly the same chiasma frequency but the means of their progenies differed by 0.11. In the analysis of variance (table 2), F for comparison of mean square between families with mean square within families

TABLE 2

Analysis of variance of indicated meiotic characters in first inbred generation families of Dactylis glomerata.

VAR. DUE TO	AVE. X-12 PER CHROMOSOME			AVE. NO. OF IV PER CELL			% OF M I WITH UNIVALENTS			% OF A I WITH LAGGARDS			% OF QUARTETS WITH MICRONUCLEI		
	D/F	MEAN SQUARE	F	D/F	MEAN SQUARE	F	D/F	MEAN SQUARE	F	D/F	MEAN SQUARE	F	D/F	MEAN SQUARE	F
Between families	1	0.0582	4.48*	7	0.6724	3.22†	7	1,807	4.95†	7	1,834	4.15†	7	3,596	10.41†
Within families	24	0.0130		75	0.2092		76	365		76	442		76	345	

* P is less than 0.05 (SNEDECOR 1938, Table 12).
† P is less than 0.01 (SNEDECOR 1938, Table 12).

was greater than F for P of 0.05. Therefore, the difference between families may be considered significant, indicating that the two parents differed in factors conditioning chiasma frequency.

QUADRIVALENT FREQUENCY

For determination of quadrivalent frequency, an average of 28 diakinesis sporocytes was examined from each plant. The data are summarized in table 3 in which the class interval of 0.29 equals the standard error of the difference for a single determination calculated from the error variance of the parental clones (MYERS 1943b). Therefore, differences greater than two class intervals probably may be considered statistically significant. Segregation for quadriva-

TABLE 3

Average number of quadrivalents per sporocyte at diakinesis in eight parental clones of Dactylis glomerata and their respective first inbred generation (I_1) *progenies.*

FAMILY NUMBER	AVE. NO. OF QUADRIV. OF		NUMBER OF I_1 PLANTS HAVING INDICATED AVERAGE NUMBER OF QUADRIVALENTS							
	PARENT	I_1	2.62* to 2.91	2.92 to 3.20	3.21 to 3.49	3.50 to 3.78	3.79 to 4.07	4.08 to 4.36	4.37 to 4.65	4.66 to 4.94
OG 48 (48)	3.00	3.66	—	—	1	3	2	—	—	—
OG 6 (12)	3.04	3.50	—	1	3	4	1	—	—	—
OG 2 (3)	3.50	3.50	3	2	5	3	4	2	1	—
OG 42 (5)	3.79	3.80	—	1	1	5	2	2	1	—
OG 48 (28)	3.92	3.99	—	—	—	3	3	1	2	—
OG 7 (20)	3.95	3.98	—	—	1	1	—	1	—	1
OG 48 (92)	4.06	4.04	—	—	2	1	4	2	3	1
OG 2 (11)	4.09	4.08	—	1	—	2	2	2	1	2

* Class interval of 0.29=S.E. of the difference for a single determination. (Based on error variance of the parental clones.)

lent frequency occurred in all progenies. The lowest number of quadrivalents was 2.62 found in one plant of family OG 2 (3), and the highest was 4.91 found in one plant of family OG 2 (11). These extremes are greater than any that have been found previously in *Dactylis glomerata*. There is no definite departure from a unimodal distribution in any of the inbred families and, consequently, no evidence for segregation of a single major factor conditioning quadrivalent frequency. The results seem more consistent with the hypothesis of segregation of two or more genes, chromosomal rearrangements, or both, with small cumulative effects upon quadrivalent formation. It should be borne in mind, however, that segregation of a single recessive factor usually would not be observed in autotetraploid populations of this size unless the parent was simplex (MYERS 1941b).

In two families, OG 48 (48) and OG 6 (12), the average quadrivalent frequency of the inbred plants significantly exceeded that of their respective parents, and these parental plants were the ones with the lowest numbers of quadrivalents. In one of these families, OG 48 (48), the chiasma frequency also was higher in the inbreds than in the parent, but the difference was not significant. In the remaining six families, the average quadrivalent frequency of the inbred plants was nearly identical with that of their respective parents. Included among these is family OG 2 (3) in which the inbreds were significantly lower than the parent in average chiasma frequency.

In the analysis of variance of the data on average number of quadrivalents (table 2), the mean square between families significantly exceeded that within families (P was less than 0.01), substantiating the hypothesis of heritable differences among parental clones for this character. Data for two of the families, OG 2 (3) and OG 48 (48), were taken from material collected in 1941, while data for the other families were taken from material collected in 1940. From examination of table 3, however, it is evident that seasonal influence was not responsible for the significant differences among families.

PERCENTAGE OF METAPHASE I SPOROCYTES WITH UNIVALENTS

The percentage of metaphase I sporocytes with one or more unpaired chromosomes was determined from an average of 142 cells for each inbred plant. The most striking feature of the results obtained with this character (table 4) was the consistently higher average of the inbred plants over that of their respective parents. In five of the progenies, the average of the inbreds was nearly double that of the parent, while in families OG 2 (11), OG 42 (5), and OG 6 (12), the frequency of univalents at metaphase I was three times as great in the inbreds as in the parents.

The frequency classes of the inbred plants were arranged with the first two intervals approximately equal to the standard error of the difference for a single determination (5.23) calculated from the error variance of the parental clones, and with the remaining class intervals approximately equal to twice that amount. The standard error does not serve as well in this case as with the

chiasma and quadrivalent frequencies. Since the error variance was an average of the variances of clones ranging in mean percentage of metaphase I univalents from three to 24, the standard error probably is too high for the plants with lower frequencies of univalents and too low for those plants with the higher percentages. No better estimate of error is available for this material, however, and it seems safe to use the standard error as calculated if the limitations are borne in mind.

It is evident from the distributions shown in table 4 that segregation for percentage of metaphase I univalents occurred in all families. In families OG 2 (11), OG 42 (5), OG 48 (92), OG 7 (20), and OG 2 (3), the majority of plants were distributed around the mean of the parent with one or more plants differing from the main group by at least two class intervals. Such distributions are suggestive of the segregation of a single major factor, the recessive condition of which results in a high frequency of sporocytes with univalents. The numbers of plants in each family are too small, however, to establish this hypothesis definitely.

It is interesting to note that even the plants with the highest incidence of sporocytes with univalents are not asynaptic in the usual sense. In most plants the majority of sporocytes with univalents had only one or two unpaired chromosomes. Three plants of family OG 2 (3) had over 80 percent of sporocytes with univalents. In one of these in which the total was 83 percent, 38 percent had a single univalent, 19 percent had two, 16 percent had three, and the highest number of univalents in any cell was six. In another plant with a

TABLE 4

Percentage of metaphase I sporocytes with univalents in eight parental clones of Dactylis glomerata and their respective first inbred generation (I_1) progenies.

FAMILY NUMBER	AVERAGE % OF MI WITH UNIVALENTS		NUMBER OF I_1 PLANTS WITH INDICATED PERCENTAGE OF MI SPOROCYTES HAVING UNIVALENTS										
	PARENT	I_1	0* to 5	6 to 10	11 to 20	21 to 30	31 to 40	41 to 50	51 to 60	61 to 70	71 to 80	81 to 90	91 to 100
OG 48 (28)	3	6	4	4	1	—	—	—	—	—	—	—	—
OG 2 (11)	4	12	3	2	4	—	1	—	—	—	—	—	—
OG 42 (5)	4	12	6	3	1	1	—	—	—	1	—	—	—
OG 48 (48)	5	10	3	—	2	1	—	—	—	—	—	—	—
OG 48 (92)	6	13	2	5	5	—	—	—	1	—	—	—	—
OG 6 (12)	9	33	1	1	1	—	2	—	2	2	—	—	—
OG 7 (20)	14	30	—	—	1	3	—	—	1	—	—	—	—
OG 2 (3)	24	42	—	—	6	2	4	1	3	1	—	2	1

* S.E. of the difference of a single determination equals 5.23. (Based on error variance of the parental clones.)

total of 83 percent there were seven percent with one, 18 percent with two, 13 percent with three, 14 percent with four, 14 percent with five, and the highest number of univalents observed was 11. One plant had a total of 97 percent of sporocytes with univalents, and in this plant, the most frequent types were those with six, seven, eight, and nine univalents while the maximum was sixteen.

In each family, most plants had a higher incidence of sporocytes with univalents than was found in their parents. Thus, even after the elimination of the plants in which the high univalent frequency seemed to result from the segregation of a major factor, the average of the inbred progenies is higher than that of the parents. Apparently, there has been segregation of recessive genes, of chromosomal rearrangements, or of both which are minor and cumulative in their effects on incidence of unpaired chromosomes at metaphase I.

There were in all families one or more inbred plants that had lower percentages of sporocytes with univalents than their parents. The occurrence of such plants suggests the possibility of obtaining increased meiotic regularity by selection. Two inbred plants were obtained with 0.78 and 1.26 percent of sporocytes with univalents, respectively. The incidence of asynapsis in these plants is as low as that found in normal plants of diploid *Lolium perenne* (MYERS 1941a).

That the differences among parental clones were heritable is shown by the analysis of variance summarized in table 2. Mean square for between families significantly exceeded mean square within families as judged by the F test (P was less than 0.01).

PERCENTAGE OF ANAPHASE I SPOROCYTES WITH LAGGING AND DIVIDING UNIVALENTS

The data on frequency of anaphase I sporocytes with lagging and equationally dividing univalents, obtained from an average of 90 sporocytes per plant, showed almost exactly the same relationships as were shown by the data on metaphase I univalents. It seems unnecessary, therefore, to present these data in tabular form. In the eight families, the inbred progenies had an average of two to three times as many laggards as their respective parents. Also, as in the case of unpaired chromosomes at metaphase I, a majority of plants in each inbred family had higher frequencies of lagging chromosomes than their parents.

The close similarity of results from anaphase I with those from metaphase I was expected, since it has been shown in several species that the unpaired chromosomes at metaphase I tend to lag and divide equationally at anaphase I. Furthermore it has been shown that metaphase I univalents are the most common source of anaphase I laggards. This relationship has been demonstrated statistically for plants of *Dactylis glomerata* by MYERS and HILL (1942) and by MYERS (1943b), and it will be shown later that a high correlation co-

efficient was obtained between the two characters with the plants studied in this investigation.

That there were heritable differences among the parental clones in percentage of chromosome lagging at anaphase I was shown by the analysis of variance summarized in table 2. The variance between families was significantly greater than the variance within families ($P < 0.01$).

PERCENTAGE OF QUARTETS WITH MICRONUCLEI

An average of 129 sporocytes per plant was examined for the determination of the frequency of quartets with micronuclei. The relationship of inbred progenies to parents for this character was very similar to those reported for metaphase I univalents and anaphase I laggards. In each family there was a similar tendency for the inbred plants to have higher frequencies of micronuclei than their parents. These relationships were expected from the known correlation of incidence of micronuclei with frequency of metaphase I univalents and with presence of laggards at anaphase I. In the analysis of variance (table 2), mean square between families was larger than mean square within families ($P < 0.01$).

CORRELATIONS AMONG CHARACTERS OF MEIOTIC BEHAVIOR

Coefficients of correlation were calculated for all combinations of the meiotic characters for which data were obtained. Coefficients were calculated separately within each inbred progeny and the average correlation coefficient was obtained by the method given by FISHER (1936) in which the individual values of r were converted to z and the weighted average of z converted back to an average value of r. These data are presented in table 5. The data from family OG 7 (20) were omitted from this table because of the low number of degrees of freedom within the family.

A correlation coefficient of +0.68 between chiasma frequency and quadrivalent frequency was obtained in both inbred progenies in which data on chiasma number were available. In family OG 2 (3) that value was significant ($P < 0.01$) as was also the average value of r. In studies of the parental clones, the value of r obtained between these two characters was +0.52 (MYERS 1943b). The difference between 0.52 and 0.68 is not significant when tested by the standard error of z (FISHER 1936).

Additional evidence regarding the relation between chiasma and quadrivalent frequency is provided by the data within plants. In each sporocyte, the chiasmata were recorded separately for the chromosomes associated as bivalents and as quadrivalents. In the 26 plants of the two inbred progenies, the average chiasma frequency of the chromosomes of the quadrivalents was in each case higher than the average for the chromosomes of the bivalents. The differences varied from 0.06 to 0.28 for the different plants. The results indicate that chiasma frequency was a limiting factor in the formation of quadriva-

TABLE 5

Correlation coefficients of characteristics of meiotic chromosomal behavior compared inter se and with selfed and open-pollinated seed set within first inbred generation families of Dactylis glomerata.

CHARACTERS	OG 48 (48)	OG 6 (12)	OG 2 (3)	OG 42 (5)	OG 48 (28)	OG 48 (92)	OG 2 (11)	AVERAGE
X-ta[1] with IV[2]	+0.68(4)[8]	—	+0.68(18)§	—	—	—	—	+0.68(21)§
X-ta with MI[3]	−0.31(4)	—	−0.87(18)§	—	—	—	—	−0.83(21)§
X-ta with AI[4]	−0.38(4)	—	−0.78(18)§	—	—	—	—	−0.74(21)§
X-ta with quartets[5]	−0.19(4)	—	−0.70(18)§	—	—	—	—	−0.65(21)§
IV with MI	−0.26(4)	−0.67(7)†	−0.56(18)§	+0.09(10)	−0.32(7)	−0.14(11)	−0.48(8)	−0.37(59)§
IV with AI	−0.35(4)	−0.61(7)*	−0.46(18)†	+0.09(10)	−0.08(7)	−0.28(11)	−0.50(8)	−0.34(59)§
IV with quartets	+0.23(4)	−0.60(7)*	−0.48(18)†	+0.10(10)	−0.04(7)	−0.13(11)	−0.37(8)	−0.26(59)†
MI with AI	+0.99(4)§	+0.99(7)§	+0.98(18)§	+0.95(10)§	+0.22(7)	+0.93(11)§	+0.89(8)§	+0.95(59)§
MI with quartets	+0.68(4)	+0.97(7)§	+0.83(18)§	+0.66(10)‡	+0.29(7)	+0.94(11)§	+0.77(8)§	+0.83(59)§
AI with quartets	+0.68(4)	+0.98(7)§	+0.76(18)§	+0.81(10)§	+0.60(7)*	+0.91(11)§	+0.93(8)§	+0.86(59)§
IV with selfed seed[6]	—	—	−0.13(17)	−0.18(9)	—	−0.11(11)	−0.06(8)	−0.12(42)
MI with selfed seed	—	—	−0.39(17)*	−0.40(9)	—	−0.25(11)	−0.46(8)	−0.37(42)‡
AI with selfed seed	—	—	−0.39(17)*	−0.50(9)	—	−0.29(11)	−0.49(8)	−0.40(42)§
Quartets with selfed seed	—	—	−0.43(17)*	−0.58(9)*	—	−0.26(11)	−0.52(8)	−0.44(42)§
IV with open seed[7]	—	—	−0.56(15)‡	+0.25(7)	—	−0.32(8)	−0.19(7)	−0.31(34)*
MI with open seed	—	—	−0.22(15)	−0.28(7)	—	−0.46(8)	−0.64(7)*	−0.37(34)†
AI with open seed	—	—	−0.31(15)	−0.47(7)	—	−0.45(8)	−0.53(7)	−0.41(34)‡
Quartets with open seed	—	—	−0.26(15)	−0.52(7)	—	−0.46(8)	−0.52(7)	−0.40(34)‡

* r exceeds value of r for P of 0.10, FISHER (1936), Table VA.
† r exceeds value of r for P of 0.05, FISHER (1936), Table VA
‡ r exceeds value of r for P of 0.02, FISHER (1936), Table VA.
§ r exceeds value of r for P of 0.01, FISHER (1936), Table VA.
[1] Average number of half-chiasmata per chromosome.
[2] Average number of quadrivalents per sporocyte.
[3] Percentage of metaphase I sporocytes with univalents.
[4] Percentage of anaphase I sporocytes with lagging and dividing univalents.
[5] Percentage of quartets with micronuclei.
[6] Number of seeds per panicle set under bag.
[7] Number of seeds per panicle set with open pollination.
[8] Numbers in parentheses are degrees of freedom.

lents in all the plants but that its effect was greater in some plants than in others. The correlation coefficient of number of half chiasmata per chromosome for the whole sporocyte with the difference in chiasma frequency between chromosomes of the bivalents and the quadrivalents was -0.59 ($P<0.01$). Apparently the differences found among plants in limitation of chiasma frequency on quadrivalent formation were partially functions of the total chiasma number. That relationship was expected. However, since total chiasma frequency accounted for only about 35 percent (r^2) of the squared variability among plants in the differences obtained, it is probable that it is not the only factor involved. If quadrivalent formation was limited differentially in the different plants by some factor other than chiasma frequency, there would be introduced a variability in the differences between chiasma frequencies of the bivalent and of the quadrivalent chromosomes which would be independent of total number of chiasmata. Thus evidence exists for factors in addition to chiasma frequency which condition differences among the inbred plants in number of quadrivalents.

Average number of chiasmata per chromosome was negatively correlated with percentage of metaphase I with univalents, percentage of anaphase I with laggards, and percentage of quartets with micronuclei (table 5). The correlation coefficients within family OG 2 (3) and also the average values of r were significant ($P<0.01$). In family OG 48 (48), the degrees of freedom were too low to provide an accurate evaluation of the correlation.

Quadrivalent frequency was negatively correlated with incidence of metaphase I univalents in six of the inbred families. In OG 42 (5), r was $+0.09$, a non-significant value. The values of r for families OG 6 (12), OG 2 (3), and average were negative and significant. Similar relationships were obtained for quadrivalent frequency correlated with anaphase I laggards and with micronuclei in the quartets, although the correlation coefficients tended to be lower in the latter two comparisons than for quadrivalent frequency with percentage of metaphase I univalents.

The correlations between metaphase I univalents, anaphase I univalents, and micronuclei in the quartets were in agreement with those reported previously for *Dactylis glomerata* by MYERS and HILL (1942) and MYERS (1943b). For the correlation of incidence of metaphase I univalents with anaphase I laggards, the average value of r was $+0.95$. For the other two comparisons of these three characters average r was $+0.83$ and $+0.86$. The only exceptions to this general relation were found in family OG 48 (28), in which the correlation coefficients of metaphase I univalents with anaphase I laggards and with micronuclei in the quartets were $+0.22$ and $+0.29$. Since r was based upon only seven degrees of freedom in this family, the discrepancy may be without significance.

CORRELATIONS BETWEEN CHARACTERS OF MEIOTIC BEHAVIOR AND SEED SET

For use in other experiments (MYERS 1942a, 1942b) the number of seeds per panicle set under parchment bag and with open pollination was determined

for the inbred plants involved in this study. Correlation coefficients were calculated for these two characters with the different characters of meiosis (table 5). Since seed set data were not obtained for all inbred plants, the degrees of freedom given in table 5 for these correlation coefficients are in some cases lower than for the comparisons between the meiotic characters. The coefficients have been omitted for three families because of the few degrees of freedom in each.

The correlation coefficients of selfed seed set with quadrivalent frequency, percentage of metaphase I sporocytes with univalents, percentage of anaphase I with laggards, and percentage of quartets with micronuclei were negative in all families and in the average. Average r was not significant for selfed seed set with quadrivalent frequency, but average r was significant for selfed seed set with incidence of metaphase I univalents ($r=-0.37$, $P<0.02$), lagging chromosomes at anaphase I ($r=-0.40$, $P<0.01$), and micronuclei in the quartets ($r=-0.44$, $P<0.01$). Open-pollinated seed set likewise was negatively correlated with the four meiotic characters, the average values of r being -0.31 ($P<0.10$), -0.37 ($P<0.05$), -0.51 ($P<0.02$), and -0.40 ($P<0.02$), respectively.

DISCUSSION

The most important feature of the data obtained in these investigations was the large increase in incidence of unpaired chromosomes at metaphase I which accompanied inbreeding in all families. The metaphase I univalents tend to lag and divide equationally at anaphase I and therefore tend to be left in the cytoplasm as micronuclei in the quartets. It was suggested by MYERS (1943b) that the unequal distribution and tendency for loss of the metaphase I univalents and their division products probably were more important factors in conditioning production of aneuploids and decreased fertility in plants of *Dactylis glomerata* than was the presence of quadrivalents *per se*. Thus inbreeding has resulted in an increase in all families of the features of meiotic irregularity which are considered of greatest importance in this species, while quadrivalent frequency on the average has been unaffected in six progenies and increased in two.

There is a striking similarity between the effects of inbreeding on meiotic irregularity found in these studies and the effects of inbreeding upon self- and open-pollinated seed set reported for *Dactylis glomerata* by MYERS (1942a). Plants of the first inbred generation set on the average only 37 percent as many seeds per panicle under bag and 62 percent as many seeds with open pollination as their parental clones. In comparison, in the present investigations the average frequency of univalents at metaphase I in the inbred progenies was increased two to threefold as compared with their parents. If there is a causal relationship between these two results, there should be a negative correlation of the frequency of asynapsis, chromosome lagging, and chromosome loss with seed set within inbred progenies. Significant negative correlation coefficients

were obtained. On the average, about 16 percent (r^2) of the squared variability in self- and open-pollinated seed set among inbred plants within families may be attributed to variation in incidence of metaphase I univalents and the resulting laggards at anaphase I and micronuclei in the quartets.

This relationship is surprisingly high when it is considered that number of seeds per panicle set under bag and with open pollination show a great amount of non-heritable variation (MYERS 1942b). Since the data for selfed seed set were obtained from three determinations per plant while those for open-pollinated seed set were obtained from a single determination, it is apparent that the values for individual inbred plants were subject to considerable inaccuracy. In studies of seed set using first inbred generation progenies including those studied in the present investigations, MYERS (1942a) found that about 38 percent and 23 percent, respectively, of the squared variability of seed set under bag and with open pollination could be attributed to heritable factors. Thus, it seems probable that a considerable proportion of the heritable variation in seed set was attributable to meiotic irregularities.

It has been pointed out (MYERS 1942a, 1942b), that number of seeds per panicle set with open pollination is conditioned by the number of florets per panicle and by the percentage fertility of the florets. Number of seeds per panicle set under bag is further influenced by a third factor, self-compatibility. With the data available to date, it has been impossible to estimate how much of the heritable variation in number of seeds per panicle may be attributed to variations in fertility and how much to the other factors. Nevertheless, it is known that great differences occur among plants of *Dactylis glomerata* in number of florets per panicle and in self-compatibility (MYERS 1942a). Variations in meiotic irregularity would condition differences in seed set by effects upon female fertility but would not be expected to influence number of florets. Neither would meiotic irregularity be expected to affect self-compatibility except in cases where pollen viability became a critical factor.

The correlation coefficient obtained between meiotic irregularities and seed set was probably lowered also by the fact that in family OG 2 (3), the one with the most degrees of freedom, seed set data were obtained in 1940, while the microsporocyte material was collected in 1941. In studies of the parental clones, MYERS (1943b) found significant year and clone×year effects upon the meiotic characters studied. Also, MYERS (1942b) found significant clone×year interactions for number of seeds per panicle set under bag. In spite of these limitations, significant correlation coefficients were obtained between incidence of metaphase I univalents, anaphase I laggards, and micronuclei in the quartets on the one hand and number of seeds per panicle on the other. It seems probable that in *Dactylis glomerata* these meiotic irregularities have a major role in conditioning variations in fertility among plants within families and the reduction in fertility which accompanies inbreeding. Experiments have been started which should provide a more critical measure of the importance of irregularities of meiosis in that regard. The significant negative correlation

coefficients of metaphase I univalents, anaphase I laggards, and micronuclei with seed set lend support to the hypothesis that these features of meiotic chromosomal behavior are more important criteria of regularity than average number of quadrivalents *per se*.

Increased meiotic irregularity accompanying inbreeding in rye has been reported by LAMM (1936). Inbred plants on the average, had lower chiasma frequencies than plants of the normal population. Chiasma frequency was inversely correlated with frequency of metaphase I univalents that lagged and divided equationally at anaphase I. Certain other results are interesting in relation to the decreased fertility which accompanied inbreeding in Dactylis. LINDSTROM and HUMPHREY (1933) found in *Lycopersicum esculentum* that the homozygous autotetraploid obtained by chromosome doubling from the haploid was extremely infertile, while tetraploids from diploid plants and from F_1 varietal crosses were fairly fertile. The diploid obtained from the haploid, on the other hand, was markedly fertile. SPARROW, RUTTLE, and NEBEL (1942), working with Antirrhinum, found that autotetraploids of varieties were lower in fertility than the hybrids between the autotetraploid varieties, although the differences could not be correlated with quadrivalent frequency. The authors concluded that sterility was a concomitant of, but was probably not conditioned by, a greater amount of pollen abortion, a significant difference in laggards at anaphase I, and a significant difference in 18:14 distributions at anaphase I. In maize, autotetraploids produced from inbreds were less fertile than those from hybrids or open-pollinated stocks (RANDOLPH 1941), but meiotic irregularities were no more common in sterile than in fertile lines (FISCHER 1941). Such results indicate that increased homozygosity tends to be accompanied by decreased fertility, at least in autopolyploids, but the behavior in Antirrhinum and maize differs from the results in *Dactylis glomerata* in that in the former species the increased homozygosity was not accompanied by increased meiotic irregularity *at least* to such a striking degree. Decreased fertility accompanying inbreeding has been encountered commonly in the naturally cross-pollinated forage species. In fact, this reduction in fertility has been one of the principal limiting factors in a successful inbreeding program with some of these species. The results with *Dactylis glomerata* raise the question of whether or not the decreased fertility has been accompanied by increased meiotic irregularity in some of these cases.

In addition to the lowered fertility caused by the greater meiotic irregularity of the inbred plants, there is also expected an increased incidence of aneuploid plants among their progenies. Thus while inbreeding might increase uniformity as a result of greater homozygosity in some progenies, in others it might increase phenotypic variability due to the occurrence of aneuploids.

An explanation of the increased incidence of unpaired chromosomes at metaphase I which accompanies inbreeding is of considerable importance. Percentage of metaphase I sporocytes with univalents was negatively correlated with chiasma frequency. Therefore, a decrease in number of chiasmata would

be accompanied by an increase in univalents at metaphase I. Data on chiasma frequency were obtained in only two progenies. In OG 2 (3), the average chiasma frequency was 0.07 per chromosome lower in the inbreds than in their parent, while the average percentage of metaphase I univalents was 18 percent higher. In the parental clones (MYERS 1943b), the regression coefficient (b) of metaphase I univalent frequency on chiasma frequency was −79.63. Using this value of b, the calculated percentage of metaphase I with univalents for the inbreds was 36 compared with the 42 percent obtained. Thus the reduced chiasma frequency did not account for all of the increase in metaphase I univalents. In OG 48 (48) the percentage of metaphase I with univalents increased from five percent for the parent to 10 percent for the average of the inbreds. The chiasma frequency of the inbreds also was higher than that of the parent, but the difference was not significant. In this case the increase of metaphase I univalents cannot be attributed to fewer chiasmata. Here the relationship between these two characters is complicated by an average increase in quadrivalent frequency for the inbreds. From the data available it seems probable that in some inbred progenies the greater incidence of metaphase I univalents may result in part but not entirely from a lower chiasma frequency, while in other progenies decreased chiasma frequency is not a factor in that regard. Data from more progenies are required to determine how generally these relationships apply. The results in these two inbred families are consistent with those obtained from the parental clones (MYERS 1943b) where it was shown by the analysis of covariance that frequency of metaphase I univalents was affected significantly by some factor or factors in addition to chiasma frequency.

In studies of plants from open-pollinated populations, MYERS and HILL (1942) and MYERS (1943b) obtained small non-significant correlation coefficients between quadrivalent frequency and percentage of metaphase I univalents. The inbred progenies in the present study, on the other hand, yielded significant negative coefficients for two progenies and for the average. These results are consistent with the hypothesis proposed by MYERS (1943b) to account for the absence of correlation between these two characters in the data obtained from the parental clones. Differences among plants in amount of chromosomal differentiation or other factors conditioning preferential prophase pairing should be less on the average within inbred families than among unrelated plants. Consequently, chiasma frequency would have a relatively greater role in conditioning differences among plants in quadrivalent frequency and incidence of metaphase I univalents within inbred families than among the parental clones. The relationship of quadrivalent frequency and incidence of metaphase I univalents to chiasma frequency is such that the two characters will tend to be negatively correlated in cases where chiasma frequency plays a dominant role (MYERS 1943b). Hence, the significant negative correlation coefficients obtained in this investigation.

The occurrence of significant differences between inbred progenies indicates

that there were heritable differences among parents in meiotic chromosomal behavior. Since the irregularities of meiosis were correlated with fertility, it should be possible to effect an appreciable increase in fertility in *Dactylis glomerata* by selection, just as it was possible to increase fertility in autotetraploid maize in that manner (RANDOLPH 1941).

SUMMARY

Chromosomal association and behavior during meiosis was studied in plants of the first inbred generation progenies of eight parental clones of *Dactylis glomerata*.

Frequency of half-chiasmata per chromosome was determined from plants of two progenies, and in one of these the average chiasma frequency of the inbred plants was significantly lower than that of the parent. In the other family the difference between progeny and parent was not significant. In six families the average number of quadrivalents per sporocyte was sensibly the same for the inbreds as for their respective parents, while in the other two families there were significant increases in quadrivalent frequency with inbreeding.

The average percentages of metaphase I sporocytes with univalents of the inbred progenies were from two to three times as great as for their respective parents in all families. Increases of similar magnitude from parent to inbred progeny were obtained for percentage of anaphase I sporocytes with lagging and dividing chromosomes and for percentage of quartets with micronuclei.

Chiasma frequency was positively correlated with quadrivalent frequency and negatively correlated with percentage of metaphase I sporocytes with univalents among plants within families. Significant negative correlation coefficients were obtained for quadrivalent frequency with incidence of metaphase I univalents, anaphase I laggards, and micronuclei within families, whereas the correlation coefficients for these characters were not significant among plants from open-pollinated populations. An explanation of this difference has been proposed. Percentages of metaphase I sporocytes with univalents, anaphase I with laggards, and quartets with micronuclei were positively and significantly correlated, *inter se*.

Number of seeds per panicle set under bag and with open pollination were both negatively correlated respectively with incidence of metaphase I univalents, anaphase I laggards, and micronuclei.

It was shown that the increased incidence of metaphase I univalents and, consequently of anaphase I laggards and micronuclei, which accompanied inbreeding could be attributed in part in one family to decreased chiasma frequency. In the other family in which data were obtained chiasma frequency was not reduced with inbreeding.

Statistically significant differences among families compared with variation within families indicated that there were heritable differences among parental clones in chiasma frequency, quadrivalent frequency, percentage of metaphase I with univalents, percentage of anaphase I with laggards, and percentage of quartets with micronuclei.

LITERATURE CITED

FISCHER, H. E., 1941 Causes of sterility in autotetraploid maize. Genetics **26**: 151.

FISHER, R. A., 1936 Statistical methods for research workers. Ed. 6. London: Oliver & Boyd.

LAMM, R., 1936 Cytological studies on inbred rye. Hereditas **22**: 217–240.

LINDSTROM, E. W., and L. M. HUMPHREY, 1933 Comparative cyto-genetic studies of tetraploid tomatoes from different origins. Genetics **18**: 193–209.

MYERS, W. M., 1941a Variations in chromosomal behavior during meiosis among plants of *Lolium perenne* L. Cytologia **11**: 388–406.

1941b Genetical consequences of chromosomal behavior in orchard grass. J. Amer. Soc. Agron. **33**: 893–900.

1942a Heritable variations in seed set under bag among plants of orchard grass, *Dactylis glomerata* L. J. Amer. Soc. Agron. **34**: 1042–1051.

1942b Analysis of non-heritable variations in seed set under bag among plants of orchard grass, *Dactylis glomerata* L. J. Amer. Soc. Agron. **34**: 1114–1124.

1943a The effects of inbreeding upon meiotic irregularity in plants of *Dactylis glomerata*. (Abstract.) Genetics **28**: 83–84.

1943b Analysis of variance and covariance of chromosomal association and behavior during meiosis in clones of *Dactylis glomerata* L. Bot. Gaz. **104**: 541–552.

MYERS, W. M., and HELEN D. HILL, 1940 Studies of chromosomal association and behavior and the occurrence of aneuploidy in the autotetraploid grass species, orchard grass, tall oat grass, and crested wheatgrass. Bot. Gaz. **102**: 236–255.

1942 Variations in chromosomal association and behavior during meiosis among plants from open-pollinated populations of *Dactylis glomerata*. Bot. Gaz. **104**: 171–177.

RANDOLPH, L. F., 1941 An evaluation of induced polyploidy as a method of breeding crop plants. Amer. Nat. **75**: 347–363.

SNEDECOR, G. W., 1938 Statistical methods. Ames, Iowa: Collegiate Press.

SPARROW, A. H., MABEL L. RUTTLE, and B. R. NEBEL, 1942 Comparative cytology of sterile intra- and fertile intervarietal tetraploids of *Antirrhinum majus* L. Amer. J. Bot. **29**: 711–715.

COMPARATIVE STUDIES OF THE CYTOGENETICAL EFFECTS OF NEUTRONS AND X-RAYS

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INTRODUCTION

THE results of a number of studies of the biological effects of neutrons and X-rays have shown that these two radiations usually differ in the efficiency with which they produce a given effect when the two are compared on the basis of equal total tissue doses of ionization. This is true for what may be referred to as general physiological responses as well as for specific cytogenetical effects. For most physiological effects, such as inhibition of growth, neutrons have been found to be more efficient than X-rays (cf. AEBERSOLD and LAWRENCE 1942). As far as cytogenetical effects are concerned, two types of experiments have been made—cytological and genetic studies of induced chromosomal rearrangements and genetic studies of lethal mutations. The genetic studies have indicated that neutrons are less efficient than X-rays in inducing lethal mutations in Drosophila (ZIMMER and TIMOFÉEF-RESSOVSKY 1938; KAUFMANN 1941b; DEMPSTER 1941; FANO 1943). For chromosomal rearrangements it is clear that neutrons are considerably more efficient than X-rays in the various plant species which have been studied (MARSHAK 1939; GILES 1940; THODAY 1942).

Differences other than the relative efficiencies of the two types of radiation have been found in the comparative experiments with induced chromosomal rearrangements in Tradescantia, the most notable being in the types of curves which show the relationship between the frequency of translocations and increasing dosage of X-rays or neutrons. It has been clearly shown (SAX 1940, 1941; RICK 1940; MARINELLI, NEBEL, GILES, and CHARLES 1942) that X-ray-induced translocations in Tradescantia increase approximately as the square of the dose when the time of irradiation is kept constant. However, for neutrons the relationship between dosage and frequency of translocations has been found to be approximately linear (GILES 1940; THODAY 1942). The present paper presents further data on this point and also evidence of other ways in which neutrons differ from X-rays in their cytogenetical effects.

MATERIALS AND METHODS

A clone of *Tradescantia paludosa* Anders. and Woodson having the haploid chromosome number of six and free from centric fragments was used as the experimental material for cytological study. Inflorescences were treated with either neutrons or X-rays, and the chromosomes were examined at metaphase or anaphase of the first post-meiotic mitosis in the microspores by means of acetocarmine smears. Studies of chromatid effects were made at from six to 28 hours following irradiation, the specific time being indicated in the tables

of experimental data. Chromosome effects were studied at five days following irradiation. The types of rearrangements induced by neutrons are the same as those produced by X-rays (GILES 1940); these have been discussed and diagrammed by SAX (1940) and THODAY (1942). In the present experiments the following terminology has been used in discussing induced aberration types (synonyms are given in parentheses): (1) Chromatid types induced at prophase—(a) chromatid terminal deletions (chromatid breaks), (b) isochromatid rejoins (chromatid dicentrics or double deletions), and (c) chromatid exchanges (rings and interchanges, both dicentric and eucentric); (2) Chromosome types induced during the resting stage—(a) chromosome terminal deletions (chromosome breaks), (b) chromosome exchanges (centric rings and dicentrics), and (c) chromosome interstitial deletions (isodiametric fragments). The class reported as chromosome rings includes only centric types, acentric rings being included in the totals for interstitial deletions. This makes possible a comparison with the results of SAX, who scores rings in this manner.

The neutrons used in these experiments were obtained from two different sources. The first source was the HARVARD cyclotron in which neutrons were produced by bombarding a beryllium target with deuterons accelerated to 11 Mev. The reaction used was:

$$Be_4^9 + D_1^2 \rightarrow B_5^{10} + n_0^1$$

and the neutrons produced were inhomogeneous with energies up to approximately 15 Mev. The second neutron source was the YALE cyclotron. Here the beryllium reaction was also used, but the deuterons were accelerated to only 3.2 Mev., the neutrons being inhomogeneous with energies up to approximately 7.5 Mev. In none of the experiments was any attempt made to exclude gamma radiation. The intensity of the irradiation was varied either by changing the distance of the inflorescences from the target or by varying the amperage of the deuteron beam. Due to the inherent difficulties in using the cyclotron, it was not always possible to control the time or the intensity of the irradiation with the desired degree of exactness, and consequently the exact dosages planned in advance in a given experiment were not always obtained.

A direct measurement of the neutron dosage was made for each exposure. Dosages were measured by means of a Victoreen r-meter with a 100 r bakelite condenser-chamber and recorded in n units, an n being defined as that amount of fast neutrons which produces the same reading with the standard Victoreen 100 r-meter as does one r of X-rays. It has been shown that in terms of ionization in tissue, an n of neutrons corresponds to between 2 and 2.5 r units of X-rays. Different 100 r chambers were used at HARVARD and YALE. A discussion of neutron dosimetry of the kind used in these experiments is given by AEBERSOLD and LAWRENCE (1942).

The X-radiations were made at the Biological Laboratories at HARVARD, using a tube operated at 160 kV and 10 mA with no filters. Dosages were measured with the 100 r dosimeter used in the neutron measurements.

The statistical problems encountered in experiments of this sort with Tradescantia have been discussed by SAX (1940, 1941). In the present data each of the experimental points was determined by analyzing from 50–200 cells on from five to ten slides. The mean of the number of samples (slides) was used as the value for a given treatment. When standard errors were calculated, the number of slides was used as the value of n in the formula $\sigma/\sqrt{n}$. In general, the standard error is between five and ten per cent of the mean.

The methods used in the comparative study of induced lethals in Dropsophila will be discussed later.

EXPERIMENTAL RESULTS

Dosage curves with neutrons

The experiments of SAX have shown that there are two types of curves relating dosage of X-rays to aberration frequency in Tradescantia when the time of irradiation is kept constant. A linear relation holds for one-hit types (isochromatid rejoins, chromatid and chromosome terminal deletions; whereas, two-hit types (chromotid exchanges and chromsome exchanges) vary as the square of the dose. The experiments of RICK (1940) indicate that the curve for chromosome interstitial deletions is intermediate between a linear and a squared relationship.

With neutrons, however, all types of rearrangements have shown an approximately linear relationship, including the two-break X-ray types. These results were first obtained using neutrons of high average energy (and at high intensities) produced by the HARVARD cyclotron (GILES 1940). The initial results have been confirmed by subsequent experiments, particularly for chromosome aberration types. Data from four separate experiments studying chromsome effects (five days after irradiation) in which the time of irradiation was kept constant are presented in table 1 (expts. A, B, CF, CK). It has also been possible to test the dosage relationship for chromosome aberrations with neutrons of lower average energy produced by the YALE cyclotron.The results of such an experiment are also presented in table 1 (expt. C-2). For both classes of neutrons it is clear that there is an approximately linear relationship with dose for chromosome exchanges (rings and dicentrics). The results of the YALE experiment (C-2) and of one of the HARVARD experiments (CF) are plotted in figure 1. The obvious difference in the slope of the two curves will be discussed later.

Interstitial deletions (isodiametric fragments) constitute the other major class of aberrations induced by irradiation during the resting stage. The dosage relation with neutrons for this type has not been previously reported. Again, it has been found (table 1, expts. CF, CK, C-2) that an approximately linear relationship holds. Since very few true terminal chromosome deletions are found in five-day material, an adequate analysis of their relationship to dose is not possible. However, the few data which are available may be interpreted as indicating a linear relationship (table 1).

Previous experiments with the various types of chromatid aberrations have

shown that all of these exhibit approximately linear relationships with neutron dose. Similar results have been obtained where chromatid effects have been analyzed in the present study.

TABLE 1

Dosage curves: time constant, intensity varied. Chromosome effects at five days.

DOSE (n)	NO. CELLS	DICENTRICS	RINGS	TOTAL	ABER./CELL	INTER. DEL.	ABER./CELL	TER. DEL.	ABER./CELL
Expt. CF (Harvard, February) Time, 15 min.									
8.0	573	36	4	40	.070±.011	38	.067	—	—
23.0	655	118	42	160	.208±.019	121	.184	—	—
44.0	591	173	56	229	.387±.016	198	.335	—	—
Expt. CK (Harvard, June) Time, 15 min.									
3.0	454	8	4	12	.028	18	.038	0	.000
7.7	540	20	14	34	.063	29	.052	1	.002
15.0	401	72	30	102	.246	79	.200	2	.005
64.0	270	161	50	211	.786	226	.830	4	.015
Expt. C-2 (Yale, October) Time, 60 min.									
3.0	1000	41	15	54	.055±.010	51	.052	2	.002
10.5	1000	98	39	137	.160±.022	159	.163	5	.005
27.0	400	125	45	170	.414±.029	222	.560	6	.015

TABLE 1 (Continued)

DOSE (n)	NO. CELLS	D	R	TOTAL	ABER./CELL
Expt. A (Harvard, February) Time, 2 min.					
10.1	564	32	12	44	.078
14.6	582	57	20	77	.132
21.0	613	86	36	122	.159
32.3	797	163	42	205	.258
Expt. B (Harvard, February) Time, 2 min.					
13.5	603	62	26	88	.147
27.0	600	86	36	122	.204
51.0	718	200	66	266	.375
89.0	633	411	137	548	.867

The X-ray results obtained by SAX and others have been interpreted as indicating that aberrations showing a linear relationship with dose are the result of a single event ("hit"); while those which vary as the square are the result of two independent events, since the chance that two or more independent events will happen together is the product of their respective chances of happening. Since with neutrons all types of aberrations are found to show a linear relationship with dose, this must mean that even those resulting from

two clearly distinct breaks are produced by a single event ("hit"). It has been suggested that the proton path is to be considered this single event—both breaks in the majority of the exchange type rearrangements being produced by single proton paths (GILES 1940; THODAY 1942).

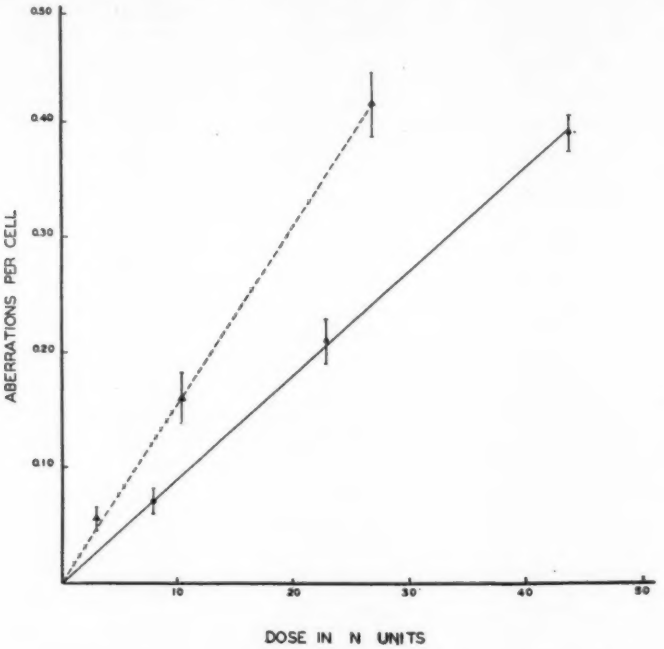

FIGURE 1.—The relation between dosage of neutrons in n units and frequencies of induced chromosome exchanges (rings and dicentrics) in Tradescantia microspores examined five days after irradiation. Results with HARVARD cyclotron (neutrons of ca 15 Mev maximum energy) in expt. CF shown by solid line; those with YALE cyclotron (neutrons of ca 7.5 Mev maximum energy) in expt. C-2 shown by broken line. Vertical lines indicate limits of standard errors.

Time-intensity factor

It is possible to test the hypothesis that neutron-induced exchange rearrangements are due to single events by experiments on the effect of the time factor similar to those which have been made with X-rays.

The existence of a time factor in the production by X-rays of exchange or two-break types of rearrangements in the developing microspores of Tradescantia has been demonstrated by SAX (1939), FABERGÉ (1940), and MARINELLI, NEBEL, GILES, CHARLES (1942). In general, experiments which prove the existence of such a factor may be classed in two separate though related categories. The first category includes those experiments in which the dosage is

varied, the relation between dosage and aberration frequency being studied. The different dosages may be obtained either by varying the duration of the irradiation, the intensity remaining constant, or by varying the intensity of irradiation, the duration remaining constant. If two curves using equal dosage points are obtained, one at constant time, the other at constant intensity, it is found that they are not identical. In both types (except perhaps at very low constant intensities (SAX 1941)) the aberration frequency is found to increase as some power greater than one of the dosage. However, with constant time this power is the square, whereas with constant intensity it is less than the square, the actual value depending on the degree of intensity.

The second category includes those experiments in which the dosage is kept constant, the effects of a single dosage administered in different ways being compared. The selected standard dosage may be given by continuous irradiation at different intensities, or by discontinuous irradiation (intermittent exposures) with a number of "rest" periods between exposures. When a certain dose is given at different intensities, the effect is found to be less at lower intensities (it shows a positive correlation with intensity) (SAX 1939; MARINELLI, et al. 1942). When a certain dose is administered in fractions, the effect is also less than when it is given as continuous irrradiation.

The simplest interpretation of these results is that restitution (refusion of broken ends in the original positions) takes place for the majority of those breaks which do not take part in an exchange rearrangement within a certain time after breakage occurs (SAX 1939). In this manner lower intensities or discontinuous irradiation would be expected to produce fewer rearrangements, since restitution of many of the initial breaks would occur before subsequent ones were available to permit new types of fusion. At high intensities or with continuous irradiation, all or most of the breaks remain open so that the maximum number of new rearrangements is favored. These results would be expected, however, only in the case of rearrangements produced by two independent events (whether these events are ionizations, or electron paths). The time factor should not be effective if the rearrangement is the result of a single event—a proton path which produces both the breaks essentially simultaneously.

Tests for the presence of a time factor with neutron-induced rearrangements were of the two general kinds just described. In the initial experiments designed to determine the relation between aberration frequency and dosage, the time of irradiation was kept constant. In some of the subsequent experiments the intensity was kept constant and the dosage increased by increasing the time. The results of two such experiments are presented in table 2. In both the relation is approximately linear for chromosome exchanges (and also for interstitial deletions in C-2) and does not differ from that obtained in experiments in which the time of irradiation was kept constant.

More definite evidence concerning the time factor may be obtained when a constant dosage is used, high versus low intensities and continuous versus intermittent irradiation being tested. Both chromosome and chromatid effects

TABLE 2

Dosage curves: time varied, intensity constant. Chromosome effects at five days.

DOSE (n)	NO. CELLS	D	R	TOTAL	ABER./ CELL	INTER. DEL.	ABER./ CELL	TER. DEL.	ABER./ CELL
Expt. CB (Harvard, December) Intensity, 15 n/min.									
18.5	709	87	30	117	.165				
28.0	697	148	48	196	.281				
69.0	725	374	134	508	.702				
Expt. C-1 (Yale, October) Intensity, 0.3 n/min.									
3	700	19	7	26	.037	45	.064	2	.003
6.3	500	26	10	36	.072	46	.092	1	.002
12.0	300	30	13	43	.143	70	.233	1	.003

were tested with the same dose at different intensities. The data for chromosome rings and dicentrics are presented in table 3. It is clear that there is no effect of the time factor with an intensity difference of 1 to 30 at approximately 26 n. For chromatid effects a direct comparison was made between X-rays and neutrons with respect to the time-intensity factor. The data are presented in table 4. The difference between the means for exchanges in the X-ray experiment is three times its standard error and is the only difference which is at all significant. For neutrons, then, there is no intensity effect for chromatid or for chromosome exchange type aberrations. The absence of any difference in the case of isochromatid rejoins with X-rays is in accord with previous experiments of SAX.

Chromatid effects were also studied in experiments using intermittent versus continuous irradiation. The data are given in table 5. In this table are included both the so-called one-hit chromatid types—simple terminal deletions and isochromatid rejoins—as well as the exchange types. It may be seen that with split doses and rest periods of 15 and 32 minutes there is no decrease in the frequency of any of the aberration types. An attempt was made to obtain an approximate base line, but the dose was less than half that for the single one-minute exposure. However, if a correction is made for this difference, it is found that the value obtained from the one-half minute exposure multiplied

TABLE 3

Intensity effect: constant dosage, intensity varied. Chromosome effects at five days.

DOSE (n)	TIME (MIN.)	NO. CELLS	D	R	TOTAL	ABER./CELL
Expt. CH (Harvard, March)						
25.5	1	615	98	43	141	.225
26.0	30	730	125	39	164	.225

TABLE 4

Intensity effect: constant dosage, intensity varied. Chromatid effects at 28 hours.

DOSE (n)	TIME (MIN.)	NO. CELLS	ISOCHROMATID REJOINS	ABER./CELL	EXCHANGES	ABER./CELL
Expt. CE (Harvard, February)						
			Neutrons			
11 n	1′	483	128	.282	66	.14
10 n	16′	375	148	.348	52	.13
			X-rays			
130 r	¾′	563	228	.400	190	.34 ± .04
130 r	13½′	475	176	.380	94	.19 ± .03

by the proper factor is approximately the same as that for the single one minute exposure, giving further evidence that restitution has not occurred during fractional dosage.

Space factors in the reunion of broken ends

The X-ray experiments of SAX (1940, 1941) have shown that reunion of broken ends does not occur at random in the nucleus, but that spatial as well as temporal limitations exist for X-ray induced two-hit breaks. Evidence of the limitations imposed by the factor of proximity in the reunion process has been obtained in two principal ways: (a) by a study of the relative frequencies of centric ring and dicentric rearrangements induced during the resting stage and (b) by a comparison of the proportions of types of chromatid aberrations induced at different times during prophase development. Comparable data are now available for neutrons.

A comparison of the dicentric:centric ring ratios for X-rays and neutrons is presented in table 6. For X-rays the ratio (based on the data of SAX 1940) is 3.29:1; for neutrons (using the total for all separate neutron experiments at HARVARD and YALE) it is 2.87:1. The difference between the ratios for the two types of radiations is highly significant ($P < .01$) as indicated by the χ^2

TABLE 5

Intensity effect: fractional dosage. Chromatid effects at 25 hours. (Harvard, April)

DOSE (n)	NO. OF EXPOSURES	DURATION OF EXPOSURES (MIN.)	REST PERIODS	TOTAL CELLS	ABERRATIONS PER CELL		
					TER. DEL.	ISOCHROMATID REJOINS	EXCHANGES
16.3	1	1′	0	616	.060	.366	.129
16.2	2	½′	15	644	.066	.402	.135
16.0	2	½′	32	599	.090	.456	.159
6.0	1	½′	0	517	.030	.168	.054

TABLE 6

Comparative frequency of dicentrics and centric rings induced by X-rays and neutrons.

		DICENTRICS	RINGS	TOTAL	RATIO
Neutrons	Harvard	3,133	1,077	4,210	2.90:1
	Yale	360	138	498	2.60:1
	Total	3,493	1,215	4,708	2.87:1
X-rays*		7,936	2,408	10,344	3.29:1
		χ^2 for Harvard vs. Yale neutrons = 1.03; P = > .05			
		χ^2 for all neutrons vs. X-rays = 11.37; P = < .01			

* From SAX (1940).

test. Furthermore there is a difference between the ratios obtained with neutrons at HARVARD compared with those at YALE, rings being even more favored in the latter experiments. This difference is not statistically significant, however, on the basis of the number of aberrations studied so far.

The results of observations at prophase of type (b) are presented in table 7. Here the X-ray data of SAX (1941) at six hours and 24 hours after irradiation are compared with neutron results at these same times. In general the results are similar, in that isochromatid rejoins are more frequent at early prophase than are the other two types. However, at later prophase, even though there is a decrease in the proportion of isochromatid rejoins, these still remain the most frequent type.

Relative efficiencies of neutrons of different energies

It has been pointed out in the discussion of the types of radiations used in these experiments that the effects of neutrons produced by two different cyclotrons were studied. Furthermore, although the reaction used to produce the neutrons—bombarding beryllium with deuterons—was the same, deuterons of different energies were used in the two cases. Consequently, the resulting neutrons were of different energies, those at HARVARD having a maximum energy of approximately 15 Mev and those at YALE having a maximum of about half this value, or 7.5 Mev.

TABLE 7

Proportions of types of chromatid aberrations induced during prophase development. Neutrons at Yale, October.

TYPES	6 HRS.		24 HRS.	
	NEUTRONS	X-RAYS*	NEUTRONS	X-RAYS*
Single terminal deletions	33%	34%	10%	23%
Isochromatid rejoins	43%	27%	60%	56%
Exchanges	24%	39%	30%	30%
No. of aberrations	122	202	283	343

* From SAX 1941.

It is clear that for neutrons of both energy ranges the relation between dosage and frequency of aberrations is a linear one, as indicated by the dosage curves. However, it appears that there is a difference in the relative efficiency of the two in producing aberrations. The neutrons of lower energy used in the YALE experiments are more efficient.

The efficiency of the neutrons in producing aberrations in any given experiment may be measured by the constant (k) in the equation: aber./cell = (Dose/k)[1], the constant being inversely related to the number of aberrations per cell. Although there is considerable variability in the several sets of experiments with dosage curves, possibly due to seasonal differences in sensitivity, slight effects of the time factor, or other causes, the value of the constant for induced chromosome rings and dicentrics is greater in all but one of the five HARVARD experiments than it is in either of the two YALE experiments (tables 1 and 2). The exceptional HARVARD experiment is number CK; it is not clear why some of the points (particularly at 15n) in this case are considerably out of line for both exchanges and deletions, although it appears that this may be due to a difference in seasonal sensitivity. In order to make a general comparison, an average k has been obtained for neutrons of the two different energies by plotting all the points in the two series of experiments, a linear relation with dose being assumed for each series. The constants obtained (YALE, k = 73, HARVARD, k = 107) indicate that the lower energy neutrons are almost 50 percent more efficient in producing rings and dicentrics, the ratio being 1.46:1

A similar comparison has been made for interstitial deletions using data from those experiments which include records for this type of aberration. Here again, the lower energy neutrons are more efficient, the ratio being 1.75:1. The data for this comparison are not very extensive, and the magnitude of the ratio is not so well established as in the case of dicentrics and rings; furthermore, this value may well be somewhat low as a general average, since one of the two HARVARD experiments included was number CK having exceptionally high values.

Adequate comparisons are not yet available for chromatid break types, but the data so far obtained indicate that here, too, lower energy neutrons are considerably more efficient. The results further suggest that the ratios vary with the type of aberration and possibly also with the stage in the nuclear cycle when irradiation takes place.

Sex-linked lethal mutations in Drosophila

The original experiments of ZIMMER and TIMOFÉEF-RESSOVSKY (1938) indicated that neutrons were less efficient than X-rays in inducing sex-linked lethal mutations in Drosophila. Since in the Tradescantia experiments with induced chromosomal aberrations the ratio of efficiency was in the opposite direction, it was early decided to perform a comparable experiment with Drosophila using the same neutron and X-ray sources as in the plant experiments. The frequency of lethals induced by X-rays and neutrons in the X chromosome

was tested by the *ClB* technique. A wild type stock which had been inbred for more than 100 generations was used. Males three to four days old were irradiated on the same day with either X-rays or neutrons at constant intensity and mated with virgin *sn/ClB* ♀ ♀. F_1 *ClB* ♀ ♀ were mated with their brother *sn* ♂ ♂ in individual bottles. Both the inbred wild type males and the signed *ClB* females were from stocks kindly furnished for the experiment by DR. SHELDON REED. In the F_2 only bottles having at least twenty-five ♀ ♀ and no ♂ ♂ were recorded as lethals. The results of the experiment are presented in table 8. Unfortunately the number of F_2 cultures obtained was

TABLE 8

Induced sex-linked lethal mutations in Drosophila.

	DOSE	NO. CULTURES	NO. LETHALS	% LETHALS	AV. k (IN %l = DOSE/k)
X-rays	1501r	114	4	3.5	417
	3003r	123	9	7.3	
Neutrons	312n	139	1	0.7	227
	649n	72	2	2.8	
	1036n	132	7	5.3	

considerably fewer than had been expected. However, it is apparent from the data available that the N/X ratio is much less for sex-linked lethals in Drosophila than for chromosomal aberrations in Tradescantia. Furthermore, if the exposure in n units is corrected to give the actual tissue dose using the factor 2.5 as found by AEBERSOLD (cf. AEBERSOLD and LAWRENCE 1942), neutrons are found to be less efficient than X-rays, the k values being 417 for X-rays and 568 for neutrons. The N/X ratio then equals 0.73. In both experiments the relation between dosage and percent mutations appears to be linear.

DISCUSSION

Nature of the interaction of neutrons and X-rays with protoplasm

It is generally agreed that X-rays and neutrons produce their effects on protoplasm as a result of the ionization or excitation of atoms and molecules. It is also clear that for both radiations this ionization is produced by secondary rather than by primary reactions. However, these secondary reactions, despite the common feature of ionization, are quite different for the two types of radiation, and it appears that the differences which have been found upon comparing X-rays and neutrons are to be ascribed to this fact. With both X-rays and neutrons the primary effect results in the production of secondary charged particles producing ionizations in the irradiated living matter. With X-rays secondary electrons result, whereas, with neutrons the majority of the secondary particles are recoil protons (hydrogen nuclei) ejected from water molecules. The tremendous difference in the masses of these two particles is associated

with a striking dissimilarity in the distribution of ion pairs along their paths in protoplasm. Protons produce a very dense ionization over a relatively short and straight path, whereas secondary electrons result in scattered clusters of a few ion pairs along a much contorted path. Consequently, if equal amounts of ionization produced in a cell or tissue by the two radiations are compared, the distribution of the ionization in the two cases will be very different. With neutrons there will be relatively few proton paths, all having ion pairs densely spaced; with X-rays, there will be many scattered, irregular electron tracks, all with rather widely spaced small clusters of ions. If these differences are thought of in molecular terms, this means that an electron might pass scores of protein molecules without producing an ion pair, whereas a proton would produce one or more ion pairs in almost every protein molecule which it traversed. A more detailed discussion of the nature of the interaction between these two kinds of radiation and matter may be found in AEBERSOLD and LAWRENCE (1942) and in LEA and CATCHESIDE (1943). Furthermore, the latter writers consider the problem of the difference between soft and hard X-rays and Gamma rays. From their discussion it is apparent that conclusions based on the absence of a wave length effect with X-rays, such as those of MULLER (1940) who concludes that chromosome breaks in Drosophila are caused by single ionizations, are not so valid as was first thought.

Having discussed briefly the manner in which X-rays and neutrons differ in their interactions with protoplasm, we may now proceed to consider whether the observed differences in the various cytogenetical effects produced by them can be explained on this basis. In none of the experiments with neutron induced chromosomal aberrations in Tradescantia has any evidence of aberration types different from those produced by X-rays been obtained. With respect to the type of aberrations produced, then, the two radiations are similar. However, as regards their relative efficiencies in producing aberrations, the nature of the relation between frequency of break types and dosage, the presence or absence of a time-intensity factor, the ratios of various aberration types at certain stages in the cell cycle and in other ways, the experimental data already presented indicate that neutrons and X-rays differ markedly.

Dosage curves and the intensity factor with neutrons and X-rays; the mechanism of rearrangement

A study of the relationship between dosage and frequency of various aberration types as well as of the intensity factor makes it possible to draw certain conclusions as to the mechanism by which these aberrations are produced by various kinds of radiations. If aberrations which require two breaks show a linear relationship with dosage and no effect of intensity, the two breaks must be produced by single ionization paths (not single ionizations, since it seems reasonable to assume that a single ionization cannot break two chromosomes at some distance apart). The original neutron experiments with Tradescantia indicated that there was a linear relationship between frequency of translocations (exchanges) and dose (GILES 1940). This relationship has been con-

firmed by subsequent experiments at both HARVARD and YALE presented in this paper, and also by THODAY (1942) at CAMBRIDGE UNIVERSITY. Furthermore, the experimental results dealing with the intensity factor also presented here indicate that there is no intensity effect for neutron-induced exchange aberrations. The absence of such an effect has been shown by studies of comparative dosage curves with either time or intensity constant, by giving single dosages at different intensities, and by the use of intermittent dosages. For both chromatid and chromosome exchanges there is no evidence that a time factor exists with neutrons. The conclusion to be drawn, then, is that these exchange aberrations are produced by two related and essentially simultaneous events: two ionizations or groups of ionizations formed by a single proton path.

For X-rays it is clear from the experiments of SAX and others that the situation with respect to exchange breaks is different. Here the relation between aberration-frequency and dosage is not linear, and there is an intensity effect. This may be taken to indicate that the two breaks which result in an exchange are the result of ionizations produced by two different electron paths.

These differences in the mechanism by which cytologically identical types of exchanges are produced by neutrons and X-rays are easily explained on the basis of the strikingly dissimilar ways in which equal total amounts of ionization produced by protons as compared with electrons are distributed in cells. As pointed out earlier, recoil protons produce densely spaced ion pairs, whereas secondary electrons produce small widely scattered clusters of ions. Consequently, with two equal dosages (of ionization), there will be considerably fewer proton than electron paths in comparable cells. And as a result the two breaks leading to an exchange will almost always be produced by a single proton path but by two independent electron paths. In terms of dosage curves exchange aberrations produced by neutrons may then be designated "one-hit" types, the proton path being the hit. This conclusion does not give any information as to the actual event which breaks the chromosome—whether it is a single ionization or a group of ionizations. This point will be discussed later.

Aberration types other than exchanges—including isochromatid rejoins and chromatid terminal deletions, and chromosome terminal and interstitial deletions induced by neutrons—show approximately linear relationships with dose and no effect of intensity and may be considered as being produced by single proton paths. This is not surprising in view of the fact that these aberration types all result either from single breaks of from two closely spaced breaks. The evidence obtained in all the neutron experiments with five-day material as well as in the X-ray results of SAX and others shows that chromosome terminal deletions are extremely rare, whereas interstitial deletions are even more abundant in terms of aberrations per cell than are exchanges (rings and dicentrics). These results do not agree with those of THODAY (1942), who finds a high frequency of chromosome breaks (=chromosome terminal deletions) and makes no mention of interstitial deletions. It seems probable that this apparent discrepancy between the HARVARD and CAMBRIDGE results is not

a real one, however, for it is clear from the figures of aberration types produced at CAMBRIDGE that interstitial deletions have not been distinguished from terminal deletions. Both metaphase plates (a) and (b) in figure 4 (THODAY 1942) contain single interstitial deletions as well as the expected acentric fragments accompanying exchanges.

For X-ray induced aberrations types other than exchanges the relationship with dosage is not always linear and depends on the type of aberration. For chromatid terminal deletions, isochromatid breaks, and chromosome terminal deletions the relation is linear, and there is no intensity effect (SAX 1940; RICK 1940). These three types, then, exhibit essentially the same dosage and intensity relationships with both X-rays and neutrons. The isochromatid rejoin is the only two-break aberration induced by X-rays whose mechanism of production is clearly similar to that of the one-hit neutron exchanges. It now seems apparent that the "hit" in the case of the chromatid dicentric is a single electron path and not a single ionization as was originally thought. This conception of the "hit" in the case of the isochromatid rejoin as a single electron path, and not a single ionization, eliminates the difficulty of explaining how a single ion pair can break two separated sister chromatids (DELBRÜCK 1940).

The nature of interstitial deletions and their relation to X-ray dose has been studied by RICK (1940). These aberrations vary as some power of dose which may be somewhat less than the square when the time is constant. Presumably they are a mixture of one- and two-hit types. With neutrons, however, these all seem to result from a single hit, which is not surprising in view of the great ionization density along a proton path.

Relative efficiencies of X-rays and neutrons

It has already been shown (MARSHAK 1939; GILES 1940; THODAY 1942) that neutrons are more efficient than X-rays in producing chromosomal aberrations. The degree of difference in efficiency of the two types of radiation may be expressed by the N/X ratio, which may be defined as the ratio obtained upon comparing the effects of equal doses (in terms of tissue ionization) of neutrons and X-rays in producing a given type of change. In the present study no special attempt was made to obtain further values for this N/X ratio for different types of rearrangements. However, it should be pointed out here that the value originally reported for the N/X ratio for isochromatid rejoins (GILES 1940) is too high. This is due to the fact that the measurements of the dosages in r units in the X-ray experiments of SAX (1940) with which comparisons were made were not exact, being somewhat less than indicated. When comparisons of the effect of dosages of neutrons and X-rays measured with the same Victoreen-meter are made—using AEBERSOLD's correction factor of 2.5 to give tissue doses—the N/X ratio for chromatid dicentrics is of the order of 5. There are also some data indicating that the N/X ratio for chromatid terminal deletions is between 3 and 4, giving further evidence that neutrons are more efficient in the actual production of chromosome breaks and do not affect the reunion process, a fact emphasized by THODAY (1942).

It has been pointed out in the previous section on dosage curves that the "hit" when used in terms of one-hit and two-hit aberrations is to be considered an electron or proton path. This conclusion does not indicate whether the actual break produced in one or more chromosomes results from one or several ionizations. The N/X ratios, however, do provide evidence on this point. Since protons produce many more ionizations in a given volume than do electrons, it follows that more ionizations will be produced within a chromosome by neutron than by X-radiation. Consequently if more than one ionization is necessary to break a chromosome, neutrons should be more efficient than X-rays, when equal doses (ionization) of the two are compared, and this is the case for all types of aberrations (within the dose range used).

This greater efficiency of neutrons over X-rays, then, indicates that more than one ionization is necessary to break a Tradescantia chromosome (GILES 1940; THODAY 1942). The results of MARSHAK (1939) with other plant genera, although not obtained in such a way as to be strictly comparable with the Tradescantia observations, also suggest that more than a single ionization is involved.

In the present study, it has been found that protons of lower average energies are more efficient in producing aberrations in Tradescantia than are those of higher average energies. The average values for all experiments with the two kinds of neutrons indicated a ratio of 1.46:1 for chromosome exchanges and of 1.75:1 for interstitial deletions. In both cases the neutron (proton) measurements were made with a standard 100r Victoreen bakelite thimble ionization chamber. As far as is known there should be no very great differences in the measurements obtained with such chambers due to the different energies of neutrons used.

It appears that this greater efficiency of lower energy protons is again due to differences in density of ionization. The density with which ion pairs are produced by a proton along its path is a function of its energy, the two being inversely related. Consequently, protons of lower average energy produce more dense ionization and are more efficient in producing chromosome breaks, according to the present evidence. In a recent paper, LEA and CATCHESIDE (1943) calculated that the number of ionizations necessary to break a Tradescantia chromosome is of the order of 17. Furthermore, they suggest that protons break a chromosome whenever they pass through one. The present evidence would seem to indicate, however, that the efficiency of an ionizing particle in breaking a chromosome is a function of ion density even with such densely ionizing particles as protons. Consequently, the probability of a break occurring when a proton traverses a chromosome would appear to be less than one, at least for high energy protons.

Since these comparisons were made using different neutron sources and were measured with different r-meters (although using standard 100r chambers whose neutron responses differ by not more than 20 percent at most, according to AEBERSOLD and LAWRENCE (1942)), it seems advisable to test the existence of an energy effect by utilizing Be neutrons of different average energies pro-

duced by one cyclotron and measured with a single 100r chamber. Such experiments are being planned.

Comparative aberration ratios with X-rays and neutrons; the effect of the space factor

SAX (1940, 1941) has discussed the nature of the evidence that with X-ray-induced breaks the reunion of broken ends does not occur at random in the nucleus, but that spatial as well as temporal limitations exist. It has already been pointed out that this evidence is obtained in two principal ways: (a) by a study of the relative frequencies of centric ring and dicentric rearrangements induced during the resting stage and (b) by a comparsion of the proportions of types of chromatid aberrations induced at different times during prophase development. On the basis of random reunion of broken ends in comparison (a), the ratio of dicentrics to centric rings should be 10:1. Actually the observed ratio for X-rays is 3.29:1, and this is good evidence that reunion is not at random. This high frequency of ring chromosomes is explained by SAX as a result of the polarized condition of the chromosomes of Tradescantia during the resting stage, exchanges between adjacent arms of the same chromosome evidently being favored.

The dicentric-ring ratio obtained for neutrons (2.86:1) is fundamentally similar to that for X-rays and indicates that the reunion of neutron-induced broken ends is also not at random. However, the difference between the two ratios is significant, and it appears that rings are even more favored with neutrons than with X-rays. As in the case of other dissimilarities between the two radiations, this one seems to be reasonably explained on the basis of ionization distribution. Apparently a densely ionizing proton will break both arms of a polarized chromosome, when these arms are within the limited distance over which refusion of broken ends can occur, in a greater proportion of cases than will two separate electron ionization paths. Furthermore, it is of interest to note that there is also a difference between the ratios when neutrons produced at HARVARD and YALE are compared. Rings are produced in still greater relative frequency with the lower energy neutrons at YALE. Even though the difference is not statistically significant on the basis of the number of observations to date, still it may well be real, and if so, it is apparently further evidence of an effect of ionization density, since protons of lower average energy at YALE produce a more dense ionization than do those of higher average energy at HARVARD as discussed previously.

The results of observations at prophase of type (b) have been discussed for X-rays by SAX (1941). The occurrence of only chromatid types during the first 25 hours after irradiation has been taken to indicate that the chromosomes must have been effectively split into sister chromatids when irradiated. Furthermore, it is clear cytologically that the chromatids are closely associated at early prophase, but become more widely separated as prophase progresses and minor coiling develops. The proportions of the various aberration types induced by X-rays during prophase vary, and these variations show an interesting correlation with chromatid behavior. A much higher proportion of

isochromatid rejoins occurs at early prophase when sister chromatids are close together, whereas single deletions and exchanges are most frequent at late prophase, when sister chromatids are more widely separated. Obviously space factors influence the reunion of X-ray-induced broken ends at prophase as in the resting stage. Again, the results with neutrons at prophase are fundamentally similar in indicating that reunion is affected by the factor of proximity. Furthermore, there are differences in the relative proportions of aberration types, and these appear to be correlated with ionization distribution. Isochromatid rejoins are more frequent at early prophase than are the other two types —as with X-rays. However, at later prophase, even though there is a decrease in the proporton of isochromatid rejoins, these still remain the most frequent type. Apparently proton ionization paths break both sister chromatids at late prophase (when they are relatively far apart) in a greater proportion of the chromosomes than do electron ionization paths. In view of the great differences in ionization density and straightness of path in the two cases, such results might well have been anticipated.

Comparative neutron and X-ray effects in Drosophila

The experiment (table 8) comparing the efficiency of neutrons and X-rays in inducing sex-linked lethal mutations in Drosophila indicates that neutrons are less efficient per ionization than are X-rays. These results agree in general with those of ZIMMER and TIMOFÉEFF-RESSOVSKY (1938), KAUFMANN (1941b), DEMPSTER (1941), and FANO (1943). The lesser efficiency of neutrons in producing sex-linked lethals has been interpreted as indicating that densely ionizing protons produce unnecessary extra ionizations within the sensitive volume of the gene (cf. LEA 1940). Calculations of this sort usually do not take into account the fact that a considerable proportion of sex-linked lethals are deficiencies. There is some cytological evidence (*vide infra*) that neutrons are actually more efficient in inducing such rearrangements than are X-rays. Consequently the genetic results with lethals may show that neutrons are much less efficient than X-rays in producing the residual gene mutations (in the restricted sense) than the data at first indicate.

It is not possible to obtain information as to the N/X ratio for effects in Tradescantia comparable to sex-linked lethals in Drosophila. However, it is clear that the same neutrons which are less efficient than X-rays in producing lethal mutations in Drosophila are more efficient in producing chromsome breaks and rearrangements in Tradescantia. These results suggest that perhaps gene mutations in the strict sense are due to single ionizations (several reasons for believing this on the basis of X-ray results alone in Drosophila are discussed by MULLER (1941)), whereas chromosome breaks require several ionizations. Such a conclusion would be more valid, however, if it were possible to compare the N/X ratio for mutations and chromosome breaks in the same organism. Such data are available only in Drosophila, and are not entirely adequate at present to permit definite conclusions to be reached. However, a brief discussion at this point seems worth while.

For X-rays there is considerable evidence in Drosophila from both genetical (MULLER 1940) and cytological studies (BAUER, DEMEREC, and KAUFMANN 1938) that the relation between dosage and frequency of gross chromosomal rearrangements (eucentric translocations and inversions) is exponential and may approach closely a two-power curve (BAUER 1939). These results have been interpreted as indicating that gross structural changes are two-hit effects, two independent ionizations being involved. For neutrons, the evidence is not nearly so complete. However, the values obtained by KAUFMANN (1941b) in an experiment comparing X-rays and neutrons clearly indicate a more gradual rise with neutrons, and the relation with dosage may indeed be linear for gross rearrangements. Thus, on the basis of present data, it appears that the relation between dosage and frequency of gross rearrangements of comparable types for both X-rays and neutrons are in essential agreement in Drosophila and Tradescantia.

It will be recalled that in order to obtain a value for the N/X ratio in Tradescantia, it is necessary to use one-hit aberration types (such as isochromatid rejoins or chromatid breaks), since only with these is the dosage-frequency relationship linear for both X-rays and neutrons. If gross rearrangements are made the basis for comparison, the value of the N/X ratio will depend on the dosage at which the comparison is made, being high at low dosages and becoming progressively less at increasing dosages. This of course results from the fact that the frequency of gross aberrations in Tradescantia increases linearly with the neutron dose, but as the square of the X-ray dose (with time constant). Since it appears that these same relations probably hold in Drosophila, the value of the N/X ratio cannot be obtained from data on gross rearrangements. Consequently it is not valid to conclude on the basis of such data (KAUFMANN 1941b) that neutrons are less efficient than X-rays in producing structural changes. In the range of dosages used in those experiments (3000 and 4000 r) it appears that X-rays are more efficient than neutrons, but if different relations to dosage obtain, the data indicate that the N/X ratio at 1000 r is about three. Unfortunately, data for lower dosages are at present not available to test this possibility.

As pointed out, the N/X ratio may be obtained directly from a comparison of isochromatid and chromatid breaks in Tradescantia regardless of the dosages used. However, such aberrations cannot be studied by the salivary gland technique in Drosophila. The nearest approach to a one-hit type for X-rays in cytological studies is the intercalary deficiencies (DEMEREC and FANO 1941), and KAUFMANN'S data suggest that neutrons are more efficient in inducing these than are X-rays. Furthermore, genetic data are available (DEMPSTER 1941; FANO 1943) which indicate that the N/X ratio for dominant lethals is greater than one and may be as much as two. It is not possible to distinguish between lethality due to one-hit and two-hit types in these experiments, but it seems probable (PONTECORVO 1942) that a considerable fraction is due to one-hit aberrations equivalent to isochromatid breaks in Tradescantia. If the two-hit aberrations could be excluded at the dosages used, the N/X

ratio for the one-hit types should be considerably higher. Consequently, it again appears that neutrons may be more efficient than X-rays in breaking chromosomes in Drosophila. The present indications, however, are that the magnitude of this efficiency is less in Drosophila than in Tradescantia. This difference may well be the result of the great difference in chromosome size between two species, the smaller chromosomes of Drosophila being broken by fewer ionizations. It seems probable that there may be a fairly direct relation between the size of a chromosome and the number of ionizations necessary to break it, and that the N/X ratio for various organisms may show a positive correlation with chromosome size.

In one respect the mechanism of rearrangement in Drosophila appears to be clearly different from that in Tradescantia. The experiments of MULLER (1940) and of KAUFMANN (1941a) show that restitution of broken ends does not occur in irradiated sperms, whereas such restitution clearly occurs in Tradescantia microspores. This dissimilarity is probably not a fundamental one, however, for it presumably results from the very dissimilar arrangement and physiological condition of the chromosomes in the two kinds of cells.

From comparative cytogenetical studies of the effects of neutrons and X-rays in Tradescantia and Drosophila it may be concluded that the "hit" which is referred to as producing one-hit or two-hit aberration types, as judged by the dosage relationship, is to be interpreted in terms of proton or electron ionization paths, rather than in terms of single ionizations or excitations. Thus it is much easier to explain one-hit X-ray types such as isochromatid rejoins in Tradescantia and deficiencies in Drosophila. Furthermore, it seems probable that in both organisms single ionizations or excitations are incapable of producing chromosome breaks, though they apparently can produce gene mutations.

SUMMARY

Comparative effects of neutrons and X-rays have been investigated in Tradescantia by a cytological analysis of chromosomal rearrangements induced by irradiation of the developing microspores. Neutrons and X-rays were found to produce the same qualitative types of rearrangements—for chromatid as well as for chromosome effects. The two radiations differ strikingly, however, in their quantitative effects. These quantitative differences are interpreted as resulting from the very dissimilar ways in which a given amount of ionization produced by the two radiations is distributed inside the treated nuclei.

For equal total tissue doses of ionization, neutrons are more efficient than X-rays in breaking chromosomes. Furthermore, under the conditions of dosimetry used in these experiments, neutrons of lower average energy (7.5 Mev) are more efficient in producing chromosomal rearrangements than are those of higher average energy (15 Mev). These results indicate that several ionizations are necessary to break a Tradescantia chromosome.

Curves relating frequency of aberrations to dosage of neutrons are approx-

imately linear for all types of rearrangements—terminal and interstitial deletions and exchanges (translocations) within the dosage range used, indicating that all these rearrangements are "one-hit" types, both breaks for most of the two-break aberrations being produced by single proton ionization paths. These results differ from those obtained with X-rays, where it is clear that exchanges are "two-hit" types, produced by two independent electron ionization paths.

Results obtained by studies of dosage curves with either time or intensity constant, by giving single doses at different intensities and by the use of intermittent doses, indicate that for both chromatid and chromosome exchanges induced by neutrons no time-intensity factor exists. This situation is contrary to that found in comparable X-ray experiments and provides further evidence that most neutron-induced exchanges are produced by single proton paths.

The ratios of certain aberration types induced during resting stage and prophase provide evidence that space factors limit the reunion of neutron-induced as well as X-ray-induced broken chromosome ends. However, the ratios with neutrons are different from those with X-rays, indicating that the denser ionization paths of neutron radiation modify the distribution of breaks and reunions within nuclei.

In Drosophila, neutrons are found to be less efficient than X-rays in inducing sex-linked lethal mutations. This effect is discussed and interpreted in comparison with the results in Tradescantia and with those obtained by previous investigators in Drosophila.

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LITERATURE CITED

AEBERSOLD, P. C., and J. H. LAWRENCE, 1942 The physiological effects of neutron rays. Annu. Rev. Physiol. **4**: 25–48.

BAUER, H., 1939 Röntgenauslösung von Chromosomen Mutationen bei *Drosophila melanogaster*. Chromosoma **1**: 343–390.

BAUER, H., M. DEMEREC, and B. P. KAUFMANN, 1938 X-ray induced chromosomal alterations in *Drosophila melanogaster*. Genetics **23**: 610–630.

DELBRÜCK, M., 1940 Radiation and the hereditary mechanism. Amer. Nat. **74**: 350–362.

DEMEREC, M., and U. FANO, 1941 Mechanism of the origin of X-ray induced notch deficiencies in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. Wash. **27**: 24–31.

DEMPSTER, E. R., 1941 Dominant vs. recessive lethal mutation. Proc. Nat. Acad. Sci. Wash. **27**: 249–250.

FABERGÉ, A. C., 1940 An experiment on chromosome fragmentation in Tradescantia by X-rays. J. Genet. **39**: 229–248.

FANO, U., 1943 Neutron-induced lethals in Drosophila. (Abstract.) Genetics **28**: 74–75.

GILES, N. H., 1940 The effect of fast neutrons on the chromosomes of Tradescantia. Proc. Nat. Acad. Sci. Wash. **26**: 567–575.

KAUFMANN, B. P., 1941a The time interval between X-radiation of sperm of Drosophila and chromosome recombination. Proc. Nat. Acad. Sci. Wash. **27**: 18–24.

1941b Induced chromosomal breaks in Drosophila. Cold Spring Harbor Symp. Quant. Biol. **9**: 82–91.

LEA, D. E., 1940 A radiation method for determining the number of genes in the X-chromosome of Drosophila. J. Genet. **39**: 181–188.

LEA, D. E., and D. G. CATCHESIDE, 1943 The mechanism of the induction by radiation of chromosome aberrations in Tradescantia. J. Genet. **44**: 216–245.

MARINELLI, L. D., B. R. NEBEL, N. H. GILES, and D. R. CHARLES, 1942 Chromosomal effects of low X-ray doses on five-day Tradescantia microspores. Amer. J. Bot. **29**: 866–874.

MARSHAK, A., 1939 Effects of fast neutrons on chromosomes in mitosis. Proc. Soc. Exp. Biol. **41**: 176–180.

MULLER, H. J., 1940 An analysis of the process of structural change in chromosomes of Drosophila. J. Genet. **40**: 1–66.

1941 Induced mutations in Drosophila. Cold Spring Harbor Symp. Quant. Biol. **9**: 151–165.

PONTECORVO, G., 1942 The problem of dominant lethals. J. Genet. **43**: 295–300.

RICK, C. M., 1940 On the nature of X-ray induced deletions in Tradescantia chromosomes. Genetics **25**: 466–482.

SAX, K., 1939 The time factor in X-ray production of chromosome aberrations. Proc. Nat. Acad. Sci. Wash. **25**: 225–233.

1940 An analysis of X-ray induced chromosomal aberrations in Tradescantia. Genetics **25**: 41–68.

1941 Types and frequencies of chromosomal aberrations induced by X-rays. Cold Spring Harbor Symp. Quant. Biol. **9**: 93–101.

THODAY, J. M., 1942 The effects of ionizing radiations on the chromosomes of *Tradescantia bracteata*. A comparison between neutrons and X-rays. J. Genet. **43**: 189–210.

ZIMMER, K. G., and N. W. TIMOFÉEOFF-RESSOVSKY, 1938 Dosimetrische und Strahlenbiologische Versuche mit schnellen Neutronen. II. Strahlentherapie **63**: 528–536.

LINKAGE STUDIES IN BARLEY[1]

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THE mode of inheritance of more than one hundred characters has been studied in barley. With a wealth of easily differentiated characters and only seven pairs of chromosomes, this crop plant provides excellent material for linkage studies. Seven linkage groups are well established, and easily differentiated viable characters are available in all but one of the linkage groups.

ROBERTSON, WIEBE, and IMMER (1941) reviewed the literature available on linkage tests up to that time and suggested appropriate symbols for the various characters. The suggested symbols will be used in this paper.

Since crossing barley is a tedious task, linkage studies are usually based on F_2 or F_3 data. The efficiency of various formulae for calculating linkage values was determined by FISHER (1936). The two methods of greatest utility are the product method and the method of maximum likelihood. FISHER and BALMUKAND (1928) and IMMER (1930) provided tables to facilitate the computation of the recombination percentage from F_2 data by the product method, the latter author providing factors also to facilitate calculating the probable errors. FISHER (1936) and IMMER (1934) discussed the method of calculating linkage from F_3 data. MATHER (1938) has given a rather complete discussion of the entire problem of measuring linkage.

MATERIAL AND METHODS

Information on the linkage relations of the following characters will be presented:

Awnless vs. awned outer glumes (*E e*). Outer glumes large and awned or hooded. Presumably the character described first by HOR (1924).

Green vs. chlorina seedlings (*F3 f3*). Seedlings chlorina in color. Viable.

Normal vs. glossy seedlings (*Gl gl*). Seedlings glossy, fully viable.

Normal vs. grandpa (*Gp gp*). Grandpa has no chlorophyll in awns or glumes, the head being white. Character described by MARTINI and HARLAN (1942).

Non-glaucous vs. glaucous sheath (*Gs gs*). Leaf sheath glaucous. Character pair can be differentiated in plants partly to fully developed.

Green vs. light green seedlings (*Lg lg*). Seedlings light green. Viable.

Green vs. light green seedlings—1 (*Lg1 lg1*). Seedlings light green, viable.

Green vs. light green seedlings—2 (*Lg2 lg2*). Seedlings light green, nonviable.

Green vs. light green seedlings—3 (*Lg3 lg3*). Seedlings light green, viable.

Normal vs. triple awned lemma (*Tr tr*). Awns or hoods on lemma of central

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florets fork to produce one central long awn, or hood, and one or two shorter awns or hoods. Referred to by MARTINI and HARLAN (1942).

Green vs. zoned leaf (*Zd zd*). Leaves of seedlings marked by transverse yellowish stripes. Viable. Plants frequently yellowish in color until almost fully developed.

The strains carrying the factors *gl*, *lg*, *lg1*, *lg2*, *lg3*, and *zd* were secured from FLOYD INGERSOLL, who, in turn, obtained them from X-rayed material stu ied in the laboratory of L. J. STADLER. The strains distinguished by *gp*, *gs*, and *e* were obtained from G. A. WIEBE as C.I. No.'s 6027, 5818, and 2523, respectively. Chlorina (Minnesota culture 89-4) was obtained from L. J. STADLER. Triple awned lemma, C.I. 6630, was secured from H. V. HARLAN.

The characters mentioned above were studied for linkage relationships with one or more of the following character pairs:

Linkage Group	Character Pair	Factor Pair
I	Non-six rowed vs. six rowed	*V v*
I	Green vs. chlorina seedlings	*F f*
I	Normal vs. virescent seedlings	*Y y*
II	Green vs. albino seedlings	*At at*
II	Black vs. white lemma and pericarp	*B b*
III	Hulled vs. naked caryopsis	*N n*
IV	Blue vs. non-blue aleurone	*Bl bl*
IV	Hooded vs. awned lemma	*K k*
V	Rough vs. smooth awn	*R r*
V	Long vs. short haired rachilla	*S s*
VI	Normal vs. xantha seedlings	*Xc xc*
VII	Normal vs. brachytic plant stature	*Br br*
VII	Green vs. chlorina seedlings	*Fc fc*
VII	Resistance vs. susceptibility to *Puccinia graminis tritici*	*T t*
VII	Normal vs. virescent seedlings	*Yc yc*
——	White vs. orange lemma	*O o*

The symbols used above are those recommended in the summary by ROBERTSON, WIEBE, and IMMER (1941).

The percentages of recombination were calculated from F_2 or F_3 data or both. The product method was used to calculate the percentage recombination when F_2 data alone were available. The method of maximum likelihood must be used with F_3 data and with F_2 also if both types of data were combined to give one recombination percentage.

EXPERIMENTAL RESULTS

Table 1 is given to facilitate calculation of the percentage of recombination from F_2 data by the product method. This table is similar to the one presented by IMMER (1930) except that the factors in the last three columns are for calculating standard errors instead of probable errors. FISHER and BALMUKAND

TABLE 1

Table to facilitate the calculation of the percentage recombination and standard errors of two simply inherited pairs of characters from F_2 data by the product method and standard error factors for backcrosses.

RECOMBINATION FRACTION	RATIO OF PRODUCTS $\frac{ad}{bc}$ (REPULSION)	RATIO OF PRODUCTS $\frac{bc}{ad}$ (COUPLING)	FACTOR TO BE DIVIDED BY $\sqrt{N}$ TO OBTAIN S.E. F_2 REPULSION	F_2 COUPLING	BACK CROSS
.00	.000000	.000000	1.0000	.0000	.0000
.01	.000200	.000136	.9999	.1001	.0995
.02	.000801	.000552	.9995	.1417	.1410
.03	.001804	.001262	.9989	.1737	.1706
.04	.003213	.002283	.9980	.2007	.1960
.05	.005031	.003629	.9969	.2246	.2179
.06	.007265	.005318	.9955	.2463	.2375
.07	.009921	.007366	.9939	.2663	.2552
.08	.01301	.009793	.9921	.2849	.2713
.09	.01653	.01262	.9900	.3025	.2862
.10	.02051	.01586	.9876	.3192	.3000
.11	.02495	.01954	.9851	.3351	.3129
.12	.02986	.02369	.9823	.3504	.3250
.13	.03527	.02832	.9793	.3651	.3363
.14	.04118	.03347	.9760	.3793	.3470
.15	.04763	.03915	.9726	.3931	.3570
.16	.05462	.04540	.9689	.4064	.3666
.17	.06218	.05225	.9650	.4194	.3756
.18	.07033	.05973	.9610	.4321	.3842
.19	.07911	.06787	.9567	.4444	.3923
.20	.08854	.07671	.9522	.4565	.4000
.21	.09865	.08628	.9475	.4684	.4073
.22	.1095	.09663	.9427	.4800	.4143
.23	.1211	.1078	.9376	.4914	.4208
.24	.1334	.1198	.9324	.5026	.4271
.25	.1467	.1328	.9270	.5136	.4330
.26	.1608	.1467	.9215	.5244	.4386
.27	.1758	.1616	.9158	.5351	.4440
.28	.1919	.1777	.9099	.5457	.4490
.29	.2089	.1948	.9039	.5560	.4538
.30	.2271	.2132	.8977	.5663	.4583
.31	.2465	.2328	.8914	.5764	.4625
.32	.2672	.2538	.8850	.5864	.4665
.33	.2892	.2763	.8784	.5963	.4702
.34	.3127	.3003	.8717	.6061	.4737
.35	.3377	.3259	.8649	.6157	.4770
.36	.3643	.3532	.8579	.6253	.4800
.37	.3927	.3823	.8509	.6348	.4828

TABLE 1 (Continued)

RECOMBINATION FRACTION	RATIO OF PRODUCTS		FACTOR TO BE DIVIDED BY $\sqrt{N}$ TO OBTAIN S.E.		
	$\frac{ad}{bc}$ (REPULSION)	$\frac{bc}{ad}$ (COUPLING)	F_2 REPULSION	F_2 COUPLING	BACK CROSS
.38	.4230	.4135	.8437	.6441	.4854
.39	.4553	.4467	.8364	.6534	.4878
.40	.4898	.4821	.8290	.6626	.4899
.41	.5266	.5199	.8215	.6717	.4918
.42	.5660	.5603	.8139	.6808	.4936
.43	.6081	.6034	.8063	.6897	.4951
.44	.6531	.6494	.7985	.6985	.4964
.45	.7013	.6985	.7906	.7073	.4975
.46	.7529	.7510	.7827	.7160	.4984
.47	.8082	.8071	.7746	.7246	.4991
.48	.8676	.8671	.7665	.7332	.4996
.49	.9314	.9313	.7583	.7416	.4999
.50	1.0000	1.0000	.7500	.7500	.5000
.51	1.0738	1.0736	.7416	.7583	.4999
.52	1.1533	1.1526	.7332	.7665	.4996
.53	1.2390	1.2373	.7246	.7746	.4991
.54	1.3316	1.3282	.7160	.7827	.4984
.55	1.4317	1.4260	.7073	.7906	.4975

(1928) first presented a table of the ratio of products ad/bc and bc/ad, where a, b, c, and d are the observed number of plants in the AB, Ab, aB, and ab phenotypes. The total number of plants is referred to in the table as N.

The factors for calculating the standard errors are equally applicable to recombination percentages calculated by the product method or by maximum likelihood.

Linkage studies in chromosome I

In table 2 are the results of linkage studies from F_2 data involving factors in chromosome I. The percentage of recombination and their standard errors were calculated by the product method, using table 1.

In the cross involving *V y Lg lg* the plants homozygous for *y y* were nonviable. With no crossing over in repulsion the expected ratio of *Lg V*:*lg V* plants is 2:1. Since the observed ratio is essentially that, the percentage recombination in this cross was essentially zero. In the cross *E e Lg lg*, the ratio of *E Lg*:*E lg* is also essentially 2:1. With a complete absence of double recessive plants a recombination percentage cannot be calculated from the remaining three classes.

The cross *V v E e* was tested in both coupling and repulsion in F_2. In the

TABLE 2

Linkage of factors in chromosome I, calculated from F_2 data.

REPULSION OR COUPLING	FACTORS		NUMBER OF PLANTS					PERCENTAGE RECOMBINATION
			X		x		SUM	
	X x	Y y	Y	y	Y	y		
Coupling	*V v*	*E e*	1221	219	246	243	1929	28± 1.2
Repulsion	*V v*	*E e*	36	12	16	2	66	36±10.6
Coupling	*V v*	*Lg lg*	320	68	70	66	524	31± 2.5
Repulsion	*V v*	*Tr tr*	431	170	164	30	795	39± 3.0
Repulsion	*Tr tr*	*Lg lg*	33	11	16	6	66	52± 9.0
Repulsion	*Tr tr*	*E e*	250	95	110	23	478	42± 3.7
Coupling	*Tr tr*	*E e*	113	30	29	16	188	40± 4.8
Repulsion	*E e*	*Lg lg*	377	191	159	0	727	
Repulsion	*Y y*	*Lg lg*	337	170		181	688	

repulsion cross the recombination fraction was .36±.106, while in the coupling cross it was .28±.012. To combine these two estimates, we may take the weighted average, weighting by the amount of information supplied by each cross. The amount of information is the reciprocal of the square of the standard error. For the repulsion cross this will be $1/(.106)^2=89$ and for the coupling cross $1/(.012)^2=6944$. The weighted average of the recombination fraction will be: $[(.36\times89)+(.28\times6944)]/(89+6944)=.28$ or 28 percent recombination. The standard error of this average percentage recombination will be the square root of the reciprocal of the total amount of information supplied by both crosses or $\sqrt{1/(89+6944)}=.012$ or 1.2 percent.

From similar calculations the combined percentage recombination of the two types of crosses in F_2 involving *Tr tr Ee* is 41±2.9 percent.

Further data may be obtained relatively easily in self-pollinated plants by growing and classifying F_3 progenies.

Symbols to represent the observed and expected frequencies for the various genotypes in F_3 are given below:

	AA	Aa	aa
BB	e p^2	f $2p(1-p)$	l $(1-p)^2$
Bb	g $2p(1-p)$	h+i $2p^2+2(1-p)^2$	m $2p(1-p)$
bb	j $(1-p)^2$	k $2p(1-p)$	n p^2

The letters e, f, g · · · n represent the observed number of F_3 lines, p represents the recombination fraction in repulsion crosses and 1−p is the recombination fraction in coupling. No separation of the Aa Bb genotype into origin as

arising from (AB×ab) or (Ab×aB) gametes will be made in any part of this study.

Formulae and methods for calculating linkage values by the method of maximum likelihood from F_3 data will now be presented. To calculate the recombination fraction from singly dominant F_3 lines—namely, Ab or aB F_2 phenotypes classified into genotypes in F_3—the formula for the Ab group is $k/p-(2j+k)/(1-p)+(j+k)2p/(1-p^2)=0$ with a standard error of $\sqrt{p(1+p)(1-p^2)/2N}$, where N is the number of such F_3 lines (see IMMER 1934). For the aB group substitute l and m for j and k, respectively, in the aforementioned formula. To facilitate calculation, in table 3 are the ratios of heterozygous to homozygous lines from singly dominant F_2 plants for different values of the recombination fraction. The amount of information per F_3 line and the factors to divide by $\sqrt{N}$ to obtain the standard error are given also.

TABLE 3

Table to facilitate calculation of recombination fraction, amount of information per F_3 line and factors for calculating standard error of singly dominant F_2 plants classified into genotypes in F_3.

RECOMBINATION FRACTION	NO. OF HETEROZYGOUS ÷HOMOZYGOUS F_3 LINES		AMOUNT OF INFORMATION PER F_3 LINE		FACTOR TO BE DIVIDED BY $\sqrt{N}$ TO OBTAIN S. E.	
	REPULSION	COUPLING	REPULSION	COUPLING	REPULSION	COUPLNG
.00	.000	∞	∞	∞	.0000	.0000
.01	.020	198.00	198.040	51.014	.0711	.1400
.02	.041	98.00	98.078	26.028	.1010	.1960
.03	.062	64.67	64.783	17.709	.1242	.2376
.04	.083	48.00	48.154	13.558	.1441	.2716
.05	.105	38.00	38.191	11.073	.1618	.3005
.06	.128	31.33	31.560	9.422	.1780	.3258
.07	.151	26.57	26.834	8.248	.1930	.3482
.08	.174	23.00	23.297	7.371	.2072	.3683
.09	.198	20.22	20.554	6.694	.2206	.3865
.10	.222	18.00	18.365	6.156	.2333	.4031
.11	.247	16.18	16.581	5.719	.2456	.4182
.12	.273	14.67	15.098	5.359	.2574	.4320
.13	.299	13.38	13.849	5.057	.2687	.4447
.14	.326	12.29	12.782	4.802	.2797	.4564
.15	.353	11.33	11.861	4.583	.2904	.4671
.16	.381	10.50	11.059	4.395	.3007	.4770
.17	.410	9.765	10.355	4.233	.3108	.4861
.18	.439	9.111	9.732	4.091	.3206	.4944
.19	.469	8.526	9.177	3.967	.3301	.5021
.20	.500	8.000	8.681	3.858	.3394	.5091
.21	.532	7.524	8.234	3.763	.3485	.5155
.22	.564	7.091	7.831	3.679	.3574	.5214
.23	.597	6.696	7.464	3.605	.3660	.5267
.24	.632	6.333	7.131	3.540	.3745	.5315
.25	.667	6.000	6.827	3.483	.3827	.5358

TABLE 3 (Continued)

RECOMBINATION FRACTION	NO. OF HETEROZYGOUS ÷HOMOZYGOUS F_3 LINES		AMOUNT OF INFORMATION PER F_3 LINE		FACTOR TO BE DIVIDED BY $\sqrt{N}$ TO OBTAIN S. E.	
	REPULSION	COUPLING	REPULSION	COUPLING	REPULSION	COUPLING
.26	.703	5.692	6.548	3.433	.3908	.5397
.27	.740	5.407	6.291	3.390	.3987	.5431
.28	.778	5.143	6.055	3.353	.4064	.5461
.29	.817	4.897	5.837	3.322	.4139	.5487
.30	.857	4.667	5.635	3.295	.4212	.5509
.31	.899	4.452	5.448	3.274	.4284	.5527
.32	.941	4.250	5.275	3.257	.4354	.5541
.33	.985	4.061	5.114	3.243	.4422	.5553
.34	1.030	3.882	4.964	3.234	.4488	.5560
.35	1.077	3.714	4.824	3.229	.4553	.5565
.36	1.125	3.556	4.693	3.227	.4616	.5566
.37	1.175	3.405	4.571	3.229	.4677	.5565
.38	1.226	3.263	4.458	3.235	.4736	.5560
.39	1.279	3.128	4.351	3.243	.4794	.5553
.40	1.333	3.000	4.252	3.255	.4850	.5543
.41	1.390	2.878	4.159	3.270	.4904	.5530
.42	1.448	2.762	4.072	3.289	.4956	.5514
.43	1.509	2.651	3.990	3.311	.5006	.5496
.44	1.571	2.546	3.914	3.335	.5054	.5476
.45	1.636	2.444	3.843	3.363	.5101	.5453
.46	1.704	2.348	3.777	3.395	.5145	.5427
.47	1.774	2.255	3.716	3.430	.5188	.5400
.48	1.846	2.167	3.658	3.468	.5228	.5370
.49	1.922	2.082	3.605	3.510	.5267	.5338
.50	2.000	2.000	3.556	3.556	.5303	.5303
.51	2.082	1.922	3.510	3.605	.5338	.5267
.52	2.167	1.846	3.468	3.658	.5370	.5228
.53	2.255	1.774	3.430	3.716	.5400	.5188
.54	2.348	1.704	3.395	3.777	.5427	.5145
.55	2.444	1.636	3.363	3.843	.5453	.5101

Three of the crosses which involved seedling characters were tested further by growing and classifying F_3 progenies from F_2 plants. The *V V* and *V v* plants can be distinguished phenotypically. The results from tests in F_3 involving linkage studies of factors in chromosome I are given in table 4.

The *Lg e* plants classified into genotypes in F_3 resulted in 141 *Lg Lg e e* and 13 *Lg lg e e* F_3 progenies. Dividing 13 by 141 gives .092, and from table 3 this ratio indicates a recombination percentage of 4. The standard error is $.1441 \div \sqrt{154} = .012$, or 1.2 percent.

When the recombination fraction is calculated from the doubly dominant

TABLE 4

Linkage of factors in chromosome I, calculated from F_2 data.

	Coupling cross			Repulsion cross			Repulsion cross	
	Lg Lg	*Lg lg*	*lg lg*		*Y Y*	*Y y*		*e e*
V v	28	35	5	*Lg Lg*	0	6	*Lg Lg*	141
V v	33	89	38	*Lg lg*	6	237	*Lg lg*	13
v v	10	42		*lg lg*	82	2		
Percentage recombination = 33 ± 3.5				Percentage recombination = 2 ± 0.5			Percentage recombination = 4 ± 1.2	

F_2 phenotypes classified into genotypes in F_3, use is made of the formula

$$\frac{2e + f + g}{p} - \frac{f + g}{1 - p} - \frac{2(h + i)(1 - 2p)}{1 - 2p + 2p^2} - \frac{(e + f + g + h + i)2p}{2 + p^2} = 0.$$

This may be solved for p by approximation after inserting the observed numbers for e, f, g, h, and i.

The amount of information per doubly dominant F_3 progeny is:

$$I = \frac{4N(2 - 6p + 3p^2 + 4p^3)}{p(1 - p)(2 + p^2)^2(1 - 2p + 2p^2)},$$

and the standard error is the square root of the reciprocal of the amount of information. In table 5 is given the amount of information per F_3 line and factors to be divided by $\sqrt{N}$ to obtain the standard error for different values of the recombination fraction.

Combining the formulae for both doubly dominant and singly dominant progenies and substituting the observed numbers for the *V v Lg lg* cross classified in F_3 leads to

$$\frac{124}{p} - \frac{68}{1 - p} - \frac{178(1 - 2p)}{1 - 2p + 2p^2} - \frac{370p}{2 + p^2} + \frac{42}{p} - \frac{62}{1 - p} + \frac{104p}{1 - p^2} + \frac{38}{p} - \frac{48}{1 - p} + \frac{86p}{1 - p^2} = 0.$$

Solving by approximation leads to a recombination percentage of 33.

The F_2 data may be combined with the F_3, since the F_2 gives information between phenotypes and the F_3 between genotypes within F_2 phenotypes. To do so the percentage recombination from F_2 must be calculated by the method of maximum likelihood. The formula is $a/(2+p^2) - (b+c)/(1-p^2) + d/p^2 = 0$, where a, b, c, and d are the observed number of plants in the AB, Ab, aB, and ab phenotypes. The amount of information will be given by: $I = 2N(1+2p^2)/(1-p^2)(2+p^2)$ and the standard error $\sqrt{(1-p^2)(2+p^2)}/2N(1+2p^2)$, where N is the number of F_2 plants. To combine F_2 and F_3 data, the formula for F_2 data is combined with that for F_3. In the case of the *V v Lg lg* linkage this consists of adding (see table 2) $320/(2+p^2) - 138/(1-p^2) + 66/p^2$ to the aforementioned formulae for doubly and singly dominant progenies classified in F_3 and solving for p. This comes to 31 percent.

TABLE 5

Table to facilitate calculation of amount of information per F_3 line and factors for calculating standard error of doubly dominant F_2 plants classified into genotypes in F_3.

RECOMBINATION FRACTION	AMOUNT OF INFORMATION PER F_3 LINE		FACTOR TO BE DIVIDED BY $\sqrt{N}$ TO OBTAIN S.E.	
	REPULSION	COUPLING	REPULSION	COUPLING
.00	∞	∞	.0000	.0000
.01	199.929	133.742	.0707	.0865
.02	99.857	67.037	.1001	.1221
.03	66.451	44.776	.1227	.1494
.04	49.709	33.625	.1418	.1725
.05	39.633	26.917	.1588	.1927
.06	32.890	22.430	.1744	.2111
.07	28.050	19.211	.1888	.2281
.08	24.399	16.785	.2024	.2441
.09	21.541	14.887	.2155	.2592
.10	19.238	13.358	.2280	.2736
.11	17.338	12.097	.2402	.2875
.12	15.741	11.037	.2520	.3010
.13	14.376	10.131	.2637	.3142
.14	13.194	9.346	.2753	.3271
.15	12.158	8.659	.2868	.3398
.16	11.241	8.049	.2983	.3525
.17	10.423	7.504	.3097	.3650
.18	9.686	7.014	.3213	.3776
.19	9.019	6.568	.3330	.3902
.20	8.410	6.161	.3488	.4029
.21	7.853	5.788	.3568	.4157
.22	7.340	5.443	.3691	.4286
.23	6.866	5.123	.3816	.4418
.24	6.427	4.826	.3945	.4552
.25	6.018	4.548	.4076	.4689
.26	5.637	4.289	.4212	.4829
.27	5.281	4.046	.4351	.4972
.28	4.949	3.817	.4495	.5118
.29	4.637	3.602	.4644	.5269
.30	4.346	3.401	.4797	.5423
.31	4.072	3.211	.4956	.5581
.32	3.816	3.032	.5119	.5743
.33	3.577	2.865	.5288	.5908
.34	3.353	2.708	.5461	.6077
.35	3.144	2.562	.5640	.6248
.36	2.949	2.425	.5823	.6421
.37	2.769	2.299	.6010	.6595
.38	2.602	2.183	.6199	.6769
.39	2.449	2.076	.6391	.6940
.40	2.308	1.980	.6582	.7107

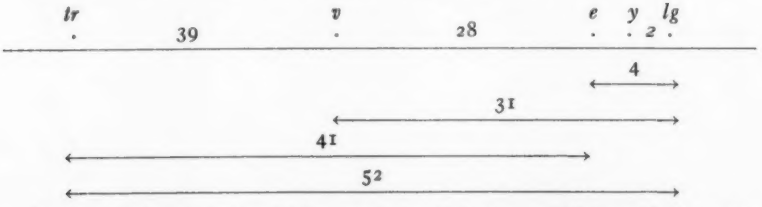
TABLE 5 (Continued)

RECOMBINATION FRACTION	AMOUNT OF INFORMATION PER F_3 LINE		FACTOR TO BE DIVIDED BY $\sqrt{N}$ TO OBTAIN S.E.	
	REPULSION	COUPLING	REPULSION	COUPLING
.41	2.181	1.893	.6772	.7268
.42	2.066	1.816	.6958	.7420
.43	1.963	1.750	.7137	.7559
.44	1.873	1.694	.7308	.7684
.45	1.794	1.648	.7465	.7790
.46	1.728	1.613	.7607	.7874
.47	1.674	1.588	.7729	.7935
.48	1.631	1.575	.7830	.7969
.49	1.600	1.572	.7906	.7976
.50	1.580	1.580	.7955	.7955
.51	1.572	1.600	.7976	.7906
.52	1.575	1.631	.7969	.7830
.53	1.588	1.674	.7935	.7729
.54	1.613	1.728	.7874	.7607
.55	1.648	1.794	.7790	.7465

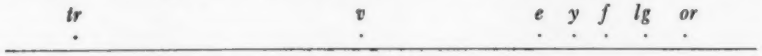
The total amount of information from all sources in the *V v Lg lg* cross will be $524/(.5764)^2=1577$ for F_2 (N=524; see table for error factor), $185\times3.211=594$ (N=185; see table 5 for I for doubly dominant progenies in F_3), and $95\times3.274=311$ (N=95; see table 3 for I) for singly dominant progenies in F_3 or a total of 2482 units of information. The standard error of the combined percentage recombination of 31 will be $\sqrt{1/2482}=.020$ or 2.0 percent.

The percentage of recombination of the factor pairs studied in this paper may be summarized as follows: $tr-v=39\pm3.0$; $tr-e=41\pm2.9$; $tr-lg=52\pm9.0$; $v-e=28\pm1.2$; $v-lg=31\pm2.5$; $e-lg=4\pm1.2$; $y-lg=2\pm0.5$.

From these data the gene order in chromosome I is:



Two other factor pairs, *Ff* for green vs. chlorina seedlings and *Or or* for green vs. orange seedlings have been located on chromosome I by ROBERTSON and COLEMAN (1940, 1942), making the known gene arrangement as follows:



Linkage studies in chromosome IV

Studies of the linkage relations of hooded vs. awned (*K k*), normal vs. zoned leaf (*Zd zd*), normal vs. glossy seedlings (*Gl gl*), green vs. light green seedlings –1 (*Lg1 lg1*), green vs. light green seedlings–2 (*Lg2 lg2*), and blue vs. colorless aleurone (*Bl bl*) were made from F_2 and F_3 data.

In table 6 are presented the F_2 data on linkage involving certain genes found to be linked with *K k* and *Bl bl* known to be in chromosome IV. The recombination percentages were calculated from the maximum likelihood formula, since these data are to be combined later with data from F_3. For such combination of F_2 and F_3 data, the maximum likelihood method must be used.

TABLE 6

Linkage of factors in chromosome IV, calculated from F_2 data.

REPULSION OR COUPLING	FACTORS		NUMBER OF PLANTS				SUM	PERCENTAGE RECOMBINATION
			X		x			
	X x	Y y	Y	y	Y	y		
Coupling	*K k*	*Zd zd*	586	8	28	140	762	5±0.8
Repulsion	*K k*	*Lg1 lg1*	272	80	97	1	450	15±4.6
Coupling	*K k*	*Lg1 lg1*	140	1	4	26	171	3±1.3
Coupling	*K k*	*Gl gl*	1100	66	65	259	1490	10±0.8
Repulsion	*K k*	*Lg2 lg2*	81		44		125	
Repulsion	*Zd zd*	*Lg1 lg1*	264	102	95	0	461	
Repulsion	*Zd zd*	*Gl gl*	454	222	233	1	910	7±3.3
Coupling	*Zd zd*	*Gl gl*	140	2	3	37	182	3±1.3
Repulsion	*Lg1 lg1*	*Gl gl*	505	198	197	1	901	11±3.3

The studies involving gene order in chromosome IV have been in progress since 1937. The data in table 1 involve data from tests made from 1939–1942 and frequently are the sums of data obtained in different experiments and in different years.

In the cross of *K K bl bl*×*k k Bl Bl* classification of the seed for blue vs. colorless aleurone into the genotypes *Bl Bl*, *Bl bl*, or *bl bl* could be made fairly accurately. Since the classification for hoods vs. awns (*K k*) was on the basis of phenotypes and blue vs. colorless aleurone (*Bl bl*) on the basis of genotypes, the data were as follows:

	Bl bl	*Bl bl*
K–	45	100
k k	15	24

The designation K– refers to the hooded F_2 phenotype and includes the *K K* and *K k* genotypes.

The percentage recombination from the ratio of *k k Bl bl*:*k k Bl Bl* geno-

types may be determined from table 3. It was found to be 44 percent. A formula for calculating the recombination fraction from the ratio of A−BB:A−Bb plants may be developed from the method of maximum likelihood. If we let the observed number of plants of A−BB and A−Bb be a′ and b′, the expected frequencies are: $N\{p(2-p)$ and $2(1-p+p^2)\}/(2+p^2)$, and the equation of maximum likelihood becomes:

$$a' \log_e \frac{p(2-p)}{2+p^2} + b' \log_e \frac{2(1-p+p^2)}{2+p^2}.$$

Maximizing this equation gives the formula for calculating p, the recombination fraction. Thus,

$$\frac{2a'(1-p)}{p(2-p)} - \frac{b'(1-2p)}{1-p+p^2} - \frac{2p(a'+b')}{2+p^2} = 0.$$

The amount of information will be given by

$$I = \frac{2N(4-8p+4p^3+p^4)}{p(2-p)(1-p+p^2)(2+p^2)^2},$$

and the standard error of p will be

$$\sqrt{\frac{p(2-p)(1-p+p^2)(2+p^2)^2}{2N(4-8p+4p^3+p^4)}}$$

In this problem $a'=45$ and $b'=100$. Substituting in the aforementioned formula leads to a percentage recombination of 45. Combining these data with those from the *k k Bl Bl*:*k k Bl bl* segregation leads to a recombination percentage between *K k* and *Bl bl* of 44 ± 6.3.

In studies of linkage involving one character which can be classified into genotypes directly, or by greenhouse tests of seedlings, the above formula for calculating linkage from doubly dominant F_2 plants in which one character is classified into genotypes and the other into phenotypes may have some appeal. The ratios of A−Bb:A−BB plants from such classification for varying values of p are given below.

p	*Ratio of A−Bb:A−BB*	*p*	*Ratio of A−Bb:A−BB*
.00	∞	.60	1.81
.10	9.58	.70	1.74
.20	4.67	.80	1.75
.30	3.10	.90	1.84
.40	2.38	1.00	2.00
.50	2.00		

The ratio of A−Bb:A−BB is seen to be the same for p=.50 and p=1.00. The amount of information obtained from such classification is greater than that obtained from ordinary F_2 data when p is less than about .36. In coupling

crosses the amount of information from doubly dominant F_2 plants classified into genotypes for one character pair only is virtually negligible. This information, however, is supplementary to that supplied by the ordinary F_2, when both factor pairs are classified into phenotypes only.

It became apparent early in the study that the gene order of the four factor pairs, *K k*, *Lg1 lg1*, *Gl gl*, and *Zd zd*, could not be established from F_2 data alone. Consequently F_3 progenies from individual F_2 plants were grown and classified. The data from this source are given in table 7.

TABLE 7

F_3 data and percentage recombination of factor pairs in chromosome IV.

Coupling cross

	Zd zd	*Zd zd*
K K	0	8
K k	0	94
k k	2	22

Percentage recombination = 7 ± 2.2

Repulsion cross

	Lg2 Lg2	*Lg2 lg2*
K K	0	0
K k	1	7
k k	41	1

Percentage recombination = 2 ± 1.4

Repulsion cross

	Gl Gl	*Gl gl*	*gl gl*
Zd Zd	0	0	195
Zd zd	2	23	6
zd zd	187	4	

Percentage recombination = 2 ± 0.5

Repulsion cross

	Lg1 Lg1	*Lg1 lg1*
K K	0	0
K k	5	118
k k	71	16

Percentage recombination = 5 ± 1.1

Repulsion cross

	Lg1 Lg1	*Lg1 lg1*
Zd Zd	0	4
Zd zd	3	264
zd zd	40	0

Percentage recombination = 1 ± 0.4

Coupling cross

	Gl Gl	*Gl gl*	*gl gl*
Zd Zd	11	1	0
Zd zd	0	77	3

Percentage recombination = 1 ± 0.9

Coupling cross

	Gl Gl	*Gl gl*
k k	3	36

Percentage recombination = 14 ± 7.3

Repulsion cross

	Gl Gl	*Gl gl*	*gl gl*
Lg1 Lg1	2	8	170
Lg1 lg1	4	304	7
lg1 lg1	69	1	

Percentage recombination = 2 ± 0.4

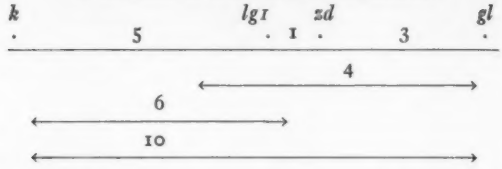
Repulsion cross

	Gl Gl	*Gl gl*	*gl gl*
Bl Bl	13	33	14
Bl bl	20	85	19
bl bl	29	35	

Percentage recombination = 36 ± 3.3

From the F_2 data in table 6 and F_3 data in table 7 may be summarized the percentage of recombination as determined from all data combined. The *Zd zd Gl gl* combination involved both F_2 and F_3 data in both repulsion and coupling. The combined percentage recombination and its error were calculated separately for the repulsion and coupling crosses by a combination of formulae for F_2 and F_3. Then the weighted average method was used to secure the combined percentage recombination for both coupling and repulsion. This came to 3 ± 0.5 percent. In a similar manner the average in the *K k Lg1 lg1* cross was 5 ± 1.0 percent. A summary of the best estimates of recombination is given: $k-zd = 6 \pm 0.8$; $k-lg1 = 5 \pm 1.0$; $k-gl = 10 \pm 0.8$; $k-lg2 = 2 \pm 1.4$; $k-bl = 44 \pm 6.3$; $zd-lg1 = 1 \pm 0.4$; $zd-gl = 3 \pm 0.5$; $lg1-gl = 4 \pm 0.6$; $gl-bl = 36 \pm 3.3$.

The gene order of the four factor pairs *K k*, *Zd zd*, *Lg1 lg1*, and *Gl gl* would appear to be:



It is clear that *k* and *gl* are the more distant genes of these four. Considering that the data came from a combination of different crosses and the errors are relatively large for the recombination percentages obtained, the order could be *k*—*lg1*—*zd*—*gl* or *k*—*zd*—*lg1*—*gl*. The factor pair *Lg2 lg2* is closely linked with *K k* but has not been tested with other genes to determine its position in the chromosome.

From the original F_2 data, obtained in 1939, it was apparent that the linkage involving *K k*, *Zd zd*, *Lg1 lg1*, and *Gl gl* was close. These early crosses involved tests of only two factor pairs at a time. Certain genotypes were selected from F_2, and further crosses were made in order that three factor pairs could be studied in a single cross. With a recombination percentage of 10 or less between the more distant of three factor pairs it seemed reasonable to assume that double crossing over would be very rare or non-existent. Thus, from the absence of certain genotypes one could infer the gene order.

One of these crosses involved the three factor pairs *k zd Lg1/K Zd lg1*. In F_3 the following genotypes were obtained.

Genotype	*No. of F_3 lines*
K K Zd zd Lg1 lg1	8
K k Zd zd Lg1 lg1	93
K k Zd zd Lg1 Lg1	1
K—*Zd Zd Lg1 lg1*	8
K—*Zd zd Lg1 Lg1*	1
k k Zd zd Lg1 lg1	8
k k zd zd Lg1 Lg1	40
k k zd zd Lg1 —	1

The order of these three genes could be: (1) *k*—*zd*—*lg1*, (2) *zd*—*k*—*lg1*, or (3) *k*—*lg1*—*zd*. The second of these three possible arrangements can probably be ruled out by the presence of the *K K Zd zd Lg1 lg1* and *k k Zd zd Lg1 lg1* genotypes. The *zd*—*k*—*lg1* order would require that 16 lines of these genotypes would need to arise through the union of two different single crossover gametes or a double and a non-crossover gamete. Double crossing over probably would not occur in a distance as short as that involved, and it is improbable that two single crossover gametes would be combined in so many lines. The order *k*—*lg1*—*zd* would require two different single crossover gametes or one double crossover and one non-crossover gamete for the formation of plants of the genotypes *K k Zd zd Lg1 Lg1* and *K*—*Zd zd Lg1 Lg1*. The gene order *k*—*zd*—*lg1* is the most probable order, since in no genotype would more than one single crossover gamete be required. The order of these three genes would appear to be *k*—*zd*—*lg1*.

In another cross involving *Zd lg1 Gl/zd Lg1 gl* the following genotypes were obtained in F_3.

Genotype			*No. of F_3 lines*
Zd Zd	*Lg1 lg1*	*Gl gl*	1
Zd Zd	*lg1 lg1*	*Gl Gl*	11
Zd zd	*Lg1 lg1*	*Gl gl*	77
Zd zd	*Lg1 Lg1*	*gl gl*	1
Zd zd	*Lg1 lg1*	*gl gl*	2
zd zd		*gl gl*	9

Of the three possible gene arrangements (1) *zd−lg1−gl*, (2) *lg1−zd−gl*, and (3) *lg1−gl−zd*, only the first is probable. The second arrangement is ruled out by the presence of the *Zd Zd Lg1 lg1 Gl gl* genotype and the third order by the *Zd zd Lg1 lg1 gl gl* genotype. Both of these could arise only through the union of two different single crossover gametes or from one non-crossover and one double crossover gamete. The order *lg1−zd−gl* is ruled out also by the presence of the *Zd zd Lg1 Lg1 gl gl* genotype which would require for its formation a double crossover gamete. The indicated order of these three genes is *zd−lg1−gl*.

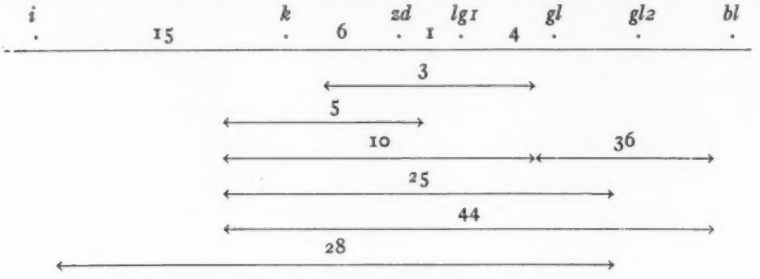
In a third cross involving *k Lg1 gl/K lg1 Gl* the following genotypes were obtained in F_3.

Genotype			*No. of F_3 lines*
K k	*Lg1 Lg1*	*gl gl*	4
K k	*Lg1 lg1*	*Gl gl*	24
K k	*Lg1 lg1*	*gl gl*	1
k k	*Lg1 Lg1*	*Gl gl*	4
k k	*Lg1 Lg1*	*gl gl*	27
k k	*Lg1 lg1*	*Gl Gl*	1
k k	*Lg1 lg1*	*Gl gl*	7

Of the three possible orders of genes: (1) *k−lg1−gl*, (2) *k−gl−lg1*, and (3) *gl−k−lg1*, the first is most reasonable. The order *k−gl−lg1* is definitely improbable because the *k k Lg1 Lg1 Gl gl* genotype would require a double crossover gamete, the *k k Lg1 lg1 Gl Gl* genotype would require a single and a double crossover gamete, and the *K k Lg1 lg1 gl gl* genotype would need to arise from two different single crossover gametes or one double and one non-crossover gamete. The order *gl−k−lg1* is ruled out for similar reasons, the genotypes *K k Lg1 Lg1 gl gl* and *k k Lg1 lg1 Gl Gl* requiring for their formation a double crossover gamete. The gene order *k−lg1−gl* requires that the one F_3 line of the genotype *k k Lg1 lg1 Gl Gl* come from two different single crossover gametes. This order is the more probable of the three possible orders.

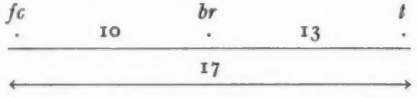
From the evidence on gene order supplied by the genotypes obtained when the four factor pairs *K k*, *Zd zd*, *Lg1 lg1*, and *Gl gl* were tested three at a time in crosses, the order of these four genes would be: *k−zd−lg1−gl*. This differs from the order indicated by an analysis of the recombination percentages. The errors of the recombination percentages were sufficiently large to allow for the correct order to be *k−zd−lg1−gl*.

ROBERTSON and COLEMAN (1942) reported glossy seedlings–2 (*Gl2 gl2*) to be linked with *K k* and *I i* with 25 and 28 percent recombination, respectively. A map of the known gene arrangement in chromosome IV would be as follows:



Linkage studies in chromosome VII

BROOKINS (1940) found the order of the three factor pairs for normal vs chlorina seedlings (*Fc fc*), normal vs. brachytic (*Br br*), and resistance vs susceptibility to *Puccinia graminis tritici* (Tt) to be



ROBERTSON, DEMING, and KOONCE (1932) found the factor pair for normal vs. virescent seedlings (*Yc yc*) to be linked with *Fc fc* with 29 percent recombination.

A cross was made involving all four factor pairs in order to determine the position of *Yc yc* in relation to the other three factor pairs. The *yc yc* genotype leads to death of the seedlings. The cross involved *Yc fc br T/yc Fc Br t*.

Since chlorina and brachytic plants suffer from reduced viability, particularly when both characters are present, the F_2 data could not be used in the analysis. BROOKINS (1940) found that reaction to race 56 of stem rust in the seedling stage was the same as reaction to a collection of races in the mature plant stage. Since the mutant characters, chlorina, brachytic, and virescent

TABLE 8

F_2 data and percentage recombination of factor pairs in chromosome VII.

Repulsion Cross			Repulsion Cross			Coupling Cross		
	Yc Yc	*Yc yc*		*Yc Yc*	*Yc yc*		*Yc Yc*	*Yc yc*
Br Br	26	159	*Fc Fc*	21	168	*T T*	10	36
Br br	169	362	*Fc fc*	170	366	*T t*	49	99
br br	73	94	*fc fc*	77	81	*t t*	33	61
Percentage recombination = 34 ± 1.8			Percentage recombination = 33 ± 1.7			Percentage recombination = 60 ± 3.4		

are seedling characters, the determination of genotypes in F_3 lines for all four character pairs was accomplished in the seedling stage in the greenhouse, using race 56 of stem rust to determine the genotype of the seedlings with respect to *P. gramini tritici*.

In table 8 are given the F_3 data involving *Yc yc* with each of the other three factor pairs.

The factor pair *Yc yc* is closest to *Fc fc* and farthest from *T t*. The gene order of the four factors known to be located in chromosome VII is:

yc		*fc*		*br*		*t*
·	33	·	10	·	13	·

Linkage studies with normal vs. grandpa

The character pair normal vs. grandpa (*Gp gp*) is due to a single factor pair. The linkage relations of this factor pair were tested in crosses with marker genes in six of the seven chromosomes. The F_2 data are given in table 9.

TABLE 9

Linkage of grandpa with factors in six chromosomes of barley, calculated from F_2 data.

REPULSION OR COUPLING	LINKAGE GROUP	FACTOR PAIR Yy	NUMBER OF PLANTS					P FROM χ^2 FOR INDEPENDENCE
			Gp		*gp*		TOTAL	
			Y	y	Y	y		
Coupling	I	*V v*	711	304	113	63	1191	.20–.10
Coupling	II	*B b*	563	200	85	54	902	<.01
Repulsion	II	*B b*	532	122	145	38	837	.70–.50
Repulsion	III	*N n*	468	168	62	22	720	.98–.95
Coupling	III	*N n*	484	170	143	40	837	.30–.20
Coupling	IV	*K k*	1375	387	210	50	2022	.50–.30
Repulsion	IV	*K k*	513	141	138	45	837	.50–.30
Repulsion	IV	*Lg1 lg1*	98	26	15	3	142	.70–.50
Repulsion	V	*R r*	487	205	96	39	827	.90–.80
Repulsion	V	*S s*	540	193	42	16	791	.90–.80
Repulsion	VII	*Br br*	568	153	84	7	812	<.01
Repulsion	VII	*Fc Fc*	580	141	89	2	812	<.01

In three crosses a significant departure from independence is indicated. In the coupling cross involving *B b* an excess of plants with white lemma and pericarp was obtained. This excess was most pronounced with the grandpa plants. The grandpa character leads to plants less vigorous and later in maturity than normals. As a result some grandpa plants may fail to turn color and be erroneously classified as white. When the cross was made in repulsion and the test made in a different season, no evidence of linkage was found.

The crosses involving *Br br* and *Fc fc* led to a low value of P in tests of inde-

TABLE 10

Percentage recombination from F_2 progenies involving grandpa.

REPULSION OR COUPLING	TESTS WITH:	NO. OF F_3 PROGENIES		PERCENTAGE RECOMBINATION
		Gp Gp	*Gp gp*	
Coupling	*v v*	61	103	54±4.0
Coupling	*b b*	9	21	46±9.9
Repulsion	*n n*	52	84	45±4.4
Coupling	*k k*	41	58	59±4.9
Repulsion	*r r*	34	53	44±5.4
Repulsion	*s s*	40	50	38±5.0

pendence. This was due to the greatly reduced survival in the double recessive class. Linkage is doubtful from these data.

Classification for normal vs. grandpa can be made in the seedling stage with a fair degree of accuracy. This is true particularly in F_3 progeny rows. In table 10 are given data from certain crosses in which singly dominant F_2 plants were classified into genotypes by growing F_3 progenies in the greenhouse and classifying in the seedling stage.

Greenhouse tests were made also of F_3 progenies of plants of the phenotypes *Br Gp* and *Fc Gp*. These data are given below:

	Repulsion Cross	
	Gp Gp	*Gp gp*
Br Br	28	43
Br br	54	87

Percentage recombination = 53±5.4

	Repulsion Cross	
	Gp Gp	*Gp gp*
Fc Fc	24	42
Fc fc	59	90

Percentage recombination = 50±5.4

No linkage is indicated with the factor pairs *Br br* and *Fc fc* in chromosome VII.

Since the F_2 data involving the coupling cross of *Gp gp* and *B b* indicated the possibility that the factor pair for normal vs. grandpa might be linked with black vs. white a second cross of *B B At At n n K K gp gp*×*b b At at N N k k Gp Gp* was grown and an extensive field test made in F_3. The data are presented in table 11.

It seems clear that *Gp gp* is independent of *B b*, *At at*, and *K k*. There is some slight evidence that *Gp gp* may be linked with *N n*. The F_3 data in table 10 indicated 45±4.4 percent recombination. In table 11 the percentage recombination was 44±3.1. Combining both sources of F_3 data gives a recombination percentage of 44±2.5.

At at was linked with *B b* with 36±2.9 percent recombination. ROBERTSON (1929) reported 22 percent recombination for these factor pairs.

Linkage studies with normal vs. chlorina seedlings (F3 f3)

The segregation of green vs. chlorina (*F3 f3*) seedlings is monohybrid. The

TABLE 11

Percentage recombination from F_2 data of cross involving normal vs. grandpa.

Repulsion Cross				Coupling Cross				Repulsion Cross			
	Gp Gp	*Gp gp*	*gp gp*		*Gp Gp*	*Gp gp*	*gp gp*		*Gp Gp*	*Gp gp*	*gp gp*
B B	48	94	22	*N N*	52	76	10	*K K*	44	65	21
B b	80	138	25	*N n*	72	128	23	*K k*	81	131	17
b b	30	48		*n n*	33	71		*k k*	33	74	
Percentage recombination = 49 ± 3.1				Percentage recombination = 44 ± 3.1				Percentage recombination = 51 ± 3.1			

Repulsion Cross				Coupling Cross			
	Gp Gp	*Gp gp*	*gp gp*		*B B*	*B b*	*b b*
At At	56	100	22	*At At*	70	77	21
At at	92	192	32	*At at*	80	161	60
Percentage recombination = 51 ± 3.4				Percentage recombination = 36 ± 2.9			

chlorina seedlings are less viable than normal green, but a high proportion develop into mature plants if growing conditions are favorable. In table 12 are presented χ^2 tests for independence with factor pairs in different linkage groups tested in F_2.

The segregations of *Xc Fc*: *Xc fc* were tested against a 3:1 ratio.

In three cases the probability from the χ^2 test indicates significant association. Calculating the percentage recombination between *N n* and *F3 f3* by the product method leads to 47 ± 0.9 percent. The ratios of *N*:*n* and *F3*:*f3* phenotypes deviate significantly from 3:1. It seems clear that these two factor pairs probably are independent.

Association is indicated between segregation for *S s* and *F3 f3*. However, the

TABLE 12

χ^2 test for independence of normal vs. chlorina (F_2 f_2) with factors in the seven linkage groups.

REPULSION OR COUPLING	LINKAGE GROUP	FACTOR PAIR Yy	NUMBER OF PLANTS					*P* FROM χ^2 TESTS FOR INDEPENDENCE
			F_2		f_2		TOTAL	
			Y	y	Y	y		
Coupling	I	*V v*	1928	699	392	166	3185	.20–.10
Coupling	II	*B b*	423	149	88	35	695	.70–.50
Coupling	III	*N n*	4061	1456	839	371	6727	<.01
Coupling	IV	*K k*	1192	373	283	101	1949	.50–.30
Repulsion	V	*R r*	1488	514	373	138	2513	.70–.50
Repulsion	V	*S s*	440	141	102	52	735	.02–.01
Repulsion	VI	*Xc xc*	1318	375	551		2244	<.01
Repulsion	VII	*Fc fc*	1029	759			1788	.50–.30
Repulsion	VII	*Br br*	797	239	219	85	1338	.10–.05
Repulsion	—	*O o*	1392	521	329	133	2375	.70–.50

percentage recombination is 56 ± 2.6 percent, indicating that these two factor pairs are independent.

The ratio of *Xc F3*: *Xc f3* plants deviated significantly from 3:1. Part of the F_2 plants of these two phenotypes were tested further in F_3 progenies. The segregations obtained were as follows:

	F3F3	*F3f3*	*f3f3*
Xc Xc	17	31	45
Xc xc	29	72	76

The percentage recombination was 47 ± 4.6.

From these data there is no clear-cut clue as to the linkage group in which *F3 f3* is contained.

Linkage studies with green vs. light green seedlings-3 (Lg3 lg3)

Light green seedlings–3 are highly viable under average field conditions. They are not so easily differentiated from normal as are light green and light green–1 seedlings. In table 13 are given χ^2 tests for independence with factor pairs in the seven linkage groups, tested in F_2.

TABLE 13

χ^2 test for independence of normal vs. light green seedlings—3 (Lg3 lg3) with factors in different linkage groups.

REPULSION OR COUPLING	LINKAGE GROUP	FACTOR PAIR *Yy*	NUMBER OF PLANTS					*P* FROM χ^2 TEST FOR INDEPENDENCE
			Lg3 Y	*Lg3 y*	*lg3 Y*	*lg3 y*	TOTAL	
Coupling	I	*V v*	750	251	193	52	1246	.30–.20
Coupling	II	*B b*	504	172	142	56	874	.50–.30
Coupling	III	*N n*	1147	386	326	120	1979	.50–.30
Coupling	IV	*K k*	484	155	115	35	789	.90–.80
Repulsion	V	*R r*	1250	427	313	130	2120	.10–.05
Repulsion	V	*S s*	472	167	106	44	789	.50–.30
Repulsion	VI	*Xc xc*	641		204		845	.70–.50
Repulsion	VII	*Br br*	247	65	39	5	356	.20–.10

From these F_2 data there is no clue of the linkage group in which *Lg3 lg3* is contained. Since classification for *Br br* and *Xc xc* can be made in the seedling stage, a further test for linkage with these two factor pairs was made in the greenhouse. The F_3 data are given below.

	Lg3 Lg3	*Lg3 lg3*	*lg3 lg3*
Br Br	8	12	0
Br br	12	31	11
br br	6	2	

Percentage recombination = 53 ± 7.8

	Lg3 Lg3	*Lg3 lg3*
Xc Xc	48	87
Xc xc	60	139

Percentage recombination = 56 ± 4.2

These F_3 data supply added information concerning the apparent independence of *Lg3 lg3* with *Br br* and *Xc xc*.

Linkage studies with glaucous sheath (Gs gs)

Classification for normal (non-glaucous) vs. glaucous sheath can be made easily in plants on which the sheath is exposed and until the plants are almost mature. In table 14 are given the results of χ^2 tests for independence, from F_2 data, with factor pairs in the first four linkage groups.

TABLE 14

χ^2 tests for independence of normal vs. glaucous sheath (Gs gs) with factor pairs in four linkage groups.

REPULSION OR COUPLING	LINKAGE GROUP	FACTOR PAIR *Y y*	NUMBER OF PLANTS					*P* FROM χ^2 TEST FOR INDEPENDENCE
			Gs Y	*Gs y*	*gs Y*	*gs y*	TOTAL	
Coupling	I	*V v*	420	113	137	46	716	.30–.20
Coupling	II	*B b*	380	153	134	49	716	.70–.50
Repulsion	III	*N n*	416	117	145	38	716	.80–.70
Coupling	IV	*K k*	371	162	142	41	716	.05–.02

There is no evidence of linkage with *V v*, *B b* or *N n*. χ^2 for independence with *K k* came to 4.28, and P is .05–.02. Calculating the percentage recombination between *Gs gs* and *K k* results in 56 ± 3.0 percent. These results suggest that *Gs gs* is not located in linkage groups I, II, III, or IV.

SUMMARY

Data on linkage relations of 11 factor pairs are presented.

Tables are given which facilitate the calculation of the percentage recombination and standard error from F_2 data and F_3 progenies of selfed singly and doubly dominant F_2 plants.

The factors for triple awned lemma (*tr*), awned outer glumes (*e*), and light green seedlings (*lg*) are located in chromosome I, making the known gene order in this chromosome *tr*–*v*–*e*–*y*–*f*–*lg*–*or*.

The factors for light green seedlings–1 (*lg1*), glossy seedlings (*gl*), and zoned leaf (*zd*) are located in chromosome IV. The known gene order in this chromosome is: *i*–*k*–*zd*–*lg1*–*gl*–*gl2*–*bl*. The factor *lg2* is close to *k*.

The order of the four genes known to be in chromosome VII was found to be: *yc*–*fc*–*br*–*t*.

Slight evidence of linkage of normal vs. grandpa (*Gp gp*) with hulled vs. naked caryopsis (*N n*) was obtained.

Green vs. chlorina seedlings (*F3 f3*) was inherited independently of one or more factor pairs in each of the seven linkage groups. The same was true of green vs. light green seedlings–3 (*Lg3 lg3*). Non-glaucous vs. glaucous sheath (*Gs gs*) was independent of *V v*, *B b*, *N n* and *K k*.

LITERATURE CITED

BROOKINS, W. W., 1940 Linkage relations of the factors determining reaction to stem rust in barley. Ph.D. Thesis. University of Minnesota.

FISHER, R. A., 1936 Statistical methods for research workers. Ed. 6. Edinburgh: Oliver and Boyd.

FISHER, R. A., and BHAI BALMUKAND, 1928 The estimation of linkage from the offspring of selfed heterozygotes. J. Genet. **20**: 79–92.

HOR, K. S., 1924 Interrelations of genetic factors in barley. Genetics **9**: 151–180.

IMMER, F. R., 1930 Formulae and tables for calculating linkage intensities. Genetics **15**: 81–98.
1934 Calculating linkage intensities from F_2 data. Genetics **19**: 119–136.

MARTINI, M. L., and H. V. HARLAN, 1942 Barley freaks. J. Hered. **33**: 339–343.

MATHER, K., 1938 The measurement of linkage in heredity. New York: Chemical Publishing Co.

ROBERTSON, D. W., 1929 Linkage studies in barley. Genetics **14**: 1–36.

ROBERTSON, D. W., and O. H. COLEMAN, 1940 The addition of two factor pairs for chlorophyll deficient seedlings to the linkage groups of barley. J. Genet. **39**: 401–410.

ROBERTSON, D. W., and O. H. COLEMAN, 1942 Location of glossy and yellow seedlings in two linkage groups. J. Amer. Soc. Agron. **34**: 1028–1034.

ROBERTSON, D. W., G. W. DEMING, and D. KOONCE, 1932 Inheritance in barley. J. Agric. Res. **44**: 445–466.

ROBERTSON, D. W., G. A. WIEBE, and F. R. IMMER, 1941 A summary of linkage studies in barley. J. Amer. Soc. Agron. **33**: 47–64.

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